PB88-103833

# Health and Environmental Effects Profile for Tetra-, Penta- and Hexachlorodibenzo-P-Dioxins

(U.S.) Environmental Protection Agency
Cincinnati, OH

Aug 84

U.S. Department of Commerce
National Technical Information Service
NTIS

PB88-103833

EPA/600/X-84/115
August 1984

# HEALTH AND ENVIRONMENTAL EFFECTS PROFILE FOR TETRA-, PENTA- AND HEXACHLORODIBENZO-P-DIOXINS

ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE
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CINCINNATI, OH 45268

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U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA 22161

## TECHNICAL REPORT DATA

*(Please read Instructions on the reverse before completing)*

**1. REPORT NO.** EPA/600/X-84/115

**2.**

**3. RECIPIENT'S ACCESSION NO.** PB88-103833/AS

**4. TITLE AND SUBTITLE**
Health and Environmental Effects Profile
For Tetra-, Penta- and Hexachlorodibenzo-p-dioxins

**5. REPORT DATE** August 1984

**6. PERFORMING ORGANIZATION CODE**

**7. AUTHOR(S)**

**8. PERFORMING ORGANIZATION REPORT NO.**

**9. PERFORMING ORGANIZATION NAME AND ADDRESS**

**10. PROGRAM ELEMENT NO.**

**11. CONTRACT/GRANT NO.**

**12. SPONSORING AGENCY NAME AND ADDRESS**
Environmental Criteria and Assessment Office
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

**13. TYPE OF REPORT AND PERIOD COVERED**

**14. SPONSORING AGENCY CODE** EPA/600/22

**15. SUPPLEMENTARY NOTES**

**16. ABSTRACT**

The Health and Environmental Effects Profile for Tetra-, Penta- and Hexachlorodibenzo-p-dioxins was prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA). Both published literature and information obtained from Agency program office files were evaluated as they pertained to potential human health, aquatic life and environmental effects of hazardous waste constituents.

Quantitative estimates have been presented provided sufficient data are available. Existing data are insufficient to determine an Acceptable Daily Intake (ADI) or a carcinogenic potency factor for Tetra-, Penta- and Hexachlorodibenzo-p-dioxins.

**17. KEY WORDS AND DOCUMENT ANALYSIS**

| a. DESCRIPTORS | b. IDENTIFIERS/OPEN ENDED TERMS | c. COSATI Field/Group |
|---|---|---|
| | | |

**18. DISTRIBUTION STATEMENT** Public

**19. SECURITY CLASS** *(This Report)* Unclassified

**20. SECURITY CLASS** *(This page)* Unclassified

**21. NO. OF PAGES** 221

**22. PRICE**

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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# PREFACE

Health and Environmental Effects Profiles (HEEPs) are prepared for the Office of Solid Waste by the Office of Health and Environmental Assessment. The HEEPs are intended to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA). Both published literature and information obtained from Agency program office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents.

Quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include, Acceptable Daily Intakes (ADIs) for chronic exposures. An ADI is defined as the amount of a chemical to which humans can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect. In the case of suspected carcinogens, ADIs are not estimated in this document series. Instead, a carcinogenic potency factor or $q_1$* is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively.

The first draft of this document was prepared by Syracuse Research Corporation under EPA Contract No. 68-03-3228. The document was subsequently revised after reviews by staff within the Office of Health and Environmental Assessment: Carcinogen Assessment Group, Reproductive Effects Assessment Group, Exposure Assessment Group, and the Environmental Criteria and Assessment Office in Cincinnati.

The HEEPs will become part of the EPA RCRA docket.

# ACKNOWLEDGEMENTS

The initial draft of this report was prepared by Syracuse Research Corporation under Contract No. 68-03-3228 for U.S. EPA's Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH. Dr. Christopher DeRosa was the Technical Project Monitor and Helen Ball was the Project Officer. The final documents in this series were prepared for the Office of Solid Waste, Washington, D.C.

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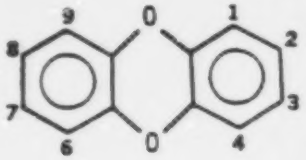
# LIST OF ABBREVIATIONS

| | |
|---|---|
| HxCDDs | hexachloro derivatives of dibenzo-p-dioxins |
| PCDDs | all polycholinated dibenzo-p-dioxins |
| PeCDDs | pentachloro derivatives of dibenzo-p-dioxins |
| 2,4,5-T | 2,4,5-trichlorophenoxyacetic acid |
| TCDDs | tetrachloro derivatives of dibenzo-p-dioxins |

# 1. INTRODUCTION

## 1.1. PHYSICAL AND CHEMICAL PROPERTIES

The polychlorodibenzo-p-dioxins (PCDDs) are a series of tricyclic aromatic compounds whose structural formula is given below:



In PCDDs substituent positions designated as 1 through 4 and 6 through 9 hold a chlorine or hydrogen atom. Tetra-, penta- and hexasubstituted dibenzo-p-dioxins have four, five and six chlorines, respectively, substituted in the two fused benzene rings. The molecular formulae, molecular weights, number of possible isomers, CAS numbers and melting points of these PCDDs are listed in Table 1-1.

As can be seen from Table 1-1, the tetra-, penta- and hexa-CDDs have 22, 14 and 10 possible isomeric forms, respectively.

The PCDDs are white solids (NRCC, 1978). Although the physical and chemical properties of pentachlorodibenzo-p-dioxins (PeCDDs) and hexachlorodibenzo-p-dioxins (HxCDDs) have not been well studied, these parameters have been more intensely studied for 2,3,7,8-TCDD. 2,3,7,8-TCDD is lipophilic, exhibiting moderate solubility in fats, oils and non-polar solvents (Table 1-2). The partition coefficient in a hexane/water system is 1000 (Matsumura and Benezet, 1973). It is only slightly soluble (0.2 μg/ℓ) in water (Crummett and Stehl, 1973). The octanol/water partition coefficient, Henry constant, and vapor pressure at 25°C were calculated to be $6.9\times10^{6}$, $2.1\times10^{-3}$ atm. $m^{3}$ $mol^{-1}$, and $10^{-6}$ mm Hg, respectively (Mabey et al., 1981). Since this vapor pressure is only estimated, this value may be inaccurate. 2,3,7,8-TCDD starts to decompose at 500°C. Decomposition is

## TABLE 1-1

### Selected Physical Properties and CAS Numbers of Some PCDDs

| Compound | Molecular Formula | Molecular Weight | Number of Possible Isomers | Melting Point[a] (°C) | CAS Number |
|---|---|---|---|---|---|
| Tetrachloro-DD (TCDDs) | $C_{12}H_4Cl_4O_2$ | 321.9 | 22 | 302-305[b] | 1746-01-6[b] |
| Pentachloro-DD (PeCDDs) | $C_{12}H_3Cl_5O_2$ | 356.5 | 14 | 195-196[c] | 39227-61-7[c] |
| | | | | 240-241[d] | 40321-76-4[d] |
| Hexachloro-DD (HxCDDs) | $C_{12}H_2Cl_6O_2$ | 390.9 | 10 | 275[e] | 39227-28-6[e] |
| | | | | 238-240[f] | 39227-62-8[f] |
| | | | | 285-286[g] | 57653-85-7[g] |
| | | | | 243-244[h] | 19408-74-3[h] |

[a] Obtained from NRCC (1978) except as in footnote b

[b] 2,3,7,8-isomer (Elvidge, 1971)

[c] 1,2,3,4,7-isomer

[d] 1,2,3,7,8-isomer

[e] 1,2,3,4,7,8-isomer

[f] 1,2,4,6,7,9-isomer

[g] 1,2,3,6,7,8-isomer

[h] 1,2,3,7,8,9-isomer

TABLE 1-2

Solubility of 2,3,7,8-TCDD in Various Solvents at 25°C*

| Solvent | Solubility (ppm) |
|---|---|
| Acetone | 110 |
| Benzene | 570 |
| Chloroform | 370 |
| o-Dichlorobenzene | 1400 |
| Methanol | 10 |
| n-Octanol | 50 |
| Water | $2 \times 10^{-4}$ |
| Lard oil | 44 |

*Source: Crummett and Stehl, 1973

reported to be virtually complete within 21 seconds at 800°C (Stehl et al., 1973). The U.S. EPA (1984) estimated the octanol/water partition coefficient to be 1.9x10$^7$. In contrast, unspecified PeCDDs and HxCDDs showed octanol/water partition coefficients of 7x10$^6$ and 4.2x10$^7$, respectively, and water solubilities of 40 and 8 ng/ℓ, respectively (NRCC, 1981a). 2,3,7,8-TCDD has a UV absorption maximum at 310 nm with a molar absorptivity of 5590 $M^{-1}$ $cm^{-1}$ in chloroform (NRCC, 1978). The UV absorption data for all PeCDDs and HxCDDs are not known but 1,2,3,7,8-PeCDD has a molar absorptivity of 6110 $M^{-1}$ $cm^{-1}$ at its maximum wavelength of 308 nm in chloroform. Similarly, 1,2,3,6,7,8-HxCDD has parameters of 5940 at 316 nm, and 1,2,3,7,8,9-HxCDD has values of 4070 at 317 nm (IARC, 1977). Thus most of these dioxins show maximum wavelengths between 308-317 nm. The infrared, fluorescence, mass and proton nuclear magnetic resonance spectra of 2,3,7,8-TCDD have been reported (IARC, 1977). The mass spectra of 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD have also been published (IARC, 1977). The response ratios in electron ionization and negative chemical ionization and fragmentation for 11 of the TCDD isomers have been reported (Rappe et al., 1983a). Specific ion monitoring of the dioxins during mass spectroscopy is the general method of quantitation after prior separation of the dioxin from a complex mixture by cleanup steps. Usually, capillary gas chromatography precedes the mass spectral detection and quantitation after the cleanup of the sample.

The available data suggest that 2,3,7,8-TCDD is stable below 500°C, and that it is rather resistant to chemical attack by acids and bases (Ayres, 1981). In organic solvents, 2,3,7,8-TCDD undergoes reductive photodechlorination at wavelengths <320 in the presence of a suitable hydrogen donor (Crosby et al., 1971; Wong and Crosby, 1978; Liberti et al., 1978). In aqueous solution, hydroxylative dechlorination probably occurs, althougn this has not been shown explicitly. Liberti et al. (1978) showed that 2,3,7,8-TCDD spread over silica gel, aluminum, glass, ceramic tile and marble in the absence of an organic solvent showed various decomposition rates upon UV irradiation. Little decomposition occurred on glass or marble, but substantial degradation occurred on silica gel and aluminum. Plimmer et al. (1973) reported that a 2,3,7,8-TCDD suspension in distilled water remained unchanged when irradiated with a sunlamp. Similarly, a thin dry film of 2,3,7,8-TCDD on a glass plate or 2,3,7,8-TCDD on dry and wet soils showed negligible photodegradation after irradiation with sunlamps (Crosby et al., 1971). In contrast, 2,3,7,8-TCDD in methanol solution, or a benzene solution of 2,3,7,8-TCDD in water stabilized by surfactant, underwent substantial photodegradation under sunlamp or sunlight irradiation (Plimmer et al., 1973; Crosby et al., 1971). 1:1 Ethyl oleate/xylene was also found to be a satisfactory H-donor stimulating the photodechlorination of 2,3,7,8-TCDD (Liberti et al., 1978). Nestrick et al. (1980) estimated the photolytic halflife of 2,3,7,8-TCDD in n-hexadecane under sunlamp irradiation to be ~57 minutes.

An increase in chlorine substitution is expected to increase the rate of photodegradation up to the hexachloro-compounds because of the red shift shown in the UV visible spectra with increasing chlorination. Crosby et al. (1971) showed, however, that although complete decomposition of 2,3,7,8-TCDD in methanol occurred in 24 hours under UV irradiation, >80% octa-CDD in methanol remained unreacted during the same period under similar irradiation conditions.

Although the degree of photolysis may be related to the extent of chlorination, positional isomerization also plays a critical and perhaps dominant part in the photolysis of higher PCDDs. In higher PCDDs, there appears to be preferential loss of chlorine from the 2, 3, 7 and 8 positions (Nestrick et al., 1980; Buser and Rappe, 1978). Thus, PCDDs with chlorine substitutions in positions 2, 3, 7 and 8 are likely to photodegrade faster than compounds not having these positional substitutions. For example, the photolysis half-life of 1,2,3,7,8-PeCDD has been estimated to be 7.6 hours in n-hexadecane solution under sunlamp irradiation (Nestrick et al., 1980). Similarly, the photolytic half-lives of 1,2,3,7,8,9-, 1,2,3,6,7,9- and 1,2,4,6,7,9-HxCDD in hexane solutions under sunlight irradiation have been determined to be 5.4, 17 and 47 hours, respectively (Dobbs and Grant, 1979). Nestrick et al. (1980) reported a half-life value of 6.8 hours for 1,2,3,6,7,8-HxCDD in n-hexadecane under sunlamp irradiation. The endproducts of photodegradation of PCDDs and the pathways of degradation in organic hydrocarbons are unknown (NRCC, 1981a), but intermediates are likely to be lower PCDDs (Buser and Rappe, 1978).

The ability of the dioxins to be perchlorinated forms the basis of an analytical method (Hutzinger et al., 1972), but cyclization of predioxins can lead to the formation of artifacts (Nilsson et al., 1974). A systematic study of the chemistry of the dioxins has not been attempted.

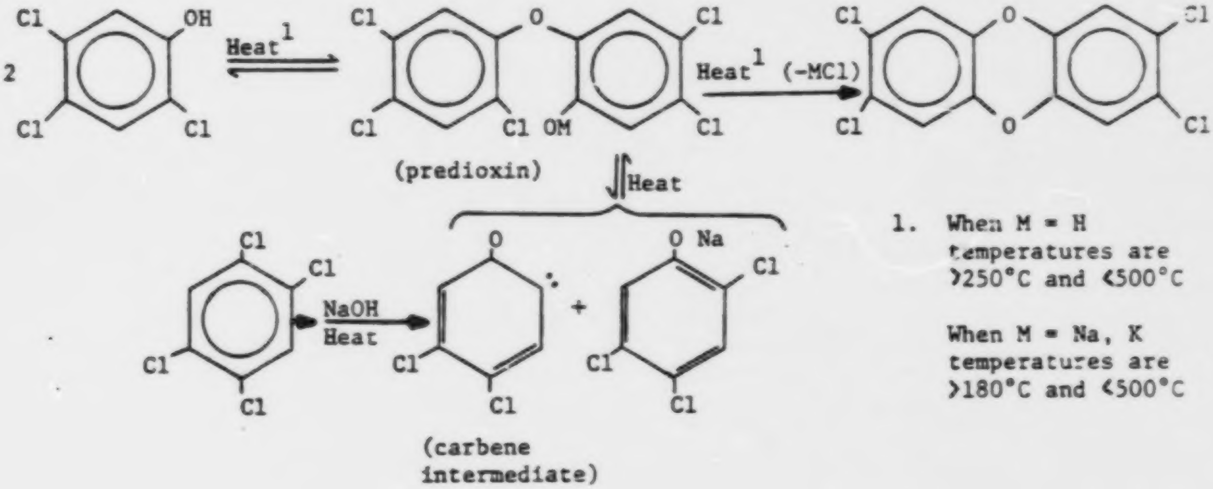
## 1.2. SOURCES OF PCDDs IN THE ENVIRONMENT

The following discussion regarding the sources of PCDDs in the environment is based primarily on a review by U.S. EPA (1980a) and an NRCC (1981a) document.

PCDDs (and chlorinated dibenzofurans) appear as inadvertant side products of many processes involving combustion or pyrolysis particularly of oxygenated chlorinated aromatics. These compounds are not manufactured deliberately by industry. They have, however, been synthesized in the laboratory.

### 1.2.1. **Manufacturing Processes.**

1.2.1.1. **CHLOROPHENOLS** -- PCDDs can be formed as by-products during the manufacture of chlorophenols. Chlorophenols are produced by two processes, namely, by chlorination of phenols and by the alkaline hydrolysis of the appropriate chlorobenzenes. The predioxin (e.g., phenoxy phenol) can be produced by heating the phenol or by condensing the quinoid form of the phenol with the sodium salt of the phenol (U.S. EPA, 1980a):


Cl
OH
2
Cl
Cl
Heat1
Cl
O
Cl
Cl
Cl OM
Cl
(predioxin)
Heat1 (-MCl)
Cl
O
Cl
Cl
O
Cl
Heat
Cl
Cl
NaOH
Heat
Cl
Cl
O
Cl
Cl
(carbene intermediate)
+
O Na
Cl
Cl
Cl

1. When M = H temperatures are >250°C and <500°C

When M = Na, K temperatures are >180°C and <500°C

A more recent review has been written by Choudhry and Hutzinger (1982). Both HxCDDs and hepta-CDDs are formed during the manufacture of tetrachloro- and pentachlorophenols by the upper reaction process. PCDDs are also expected to be formed during the hydrolysis of polychlorinated benzenes to produce phenols. The amounts of PCDDs in commercial chlorophenols vary according to manufacturing process and conditions. For example, the amounts of total TCDDs, PeCDD and HxCDDs in various commercial pentachlorophenols have been found to vary from 0.003-0.1, 0.03-0.5 and 1.7-44.0 mg/kg, respectively (NRCC, 1981a), although other congeners are not given.

1.2.1.2. **CHLOROPHENOL DERIVATIVES** -- PCDDs are produced as contaminants during the manufacture of commonly used chlorophenol derivatives such as 2,4-D, 2,4,5-T and hexachlorophene by mechanisms hypothesized to be similar to those discussed in the case of chlorophenols. The amounts of 1,3,6,8- and 1,3,7,9-TCDD in commercial isooctyl-, mixed butyl- and propylene glycol butyl ether esters of 2,4-D varied from nondetectable to 8.7 mg/kg (Cochrane et al., 1981). Certain batches of Agent Orange, which is a 1:1 mixture of butyl esters of 2,4-D and 2,4,5-T, have been shown to contain 2,3,7,8-TCDD in quantities in excess of 32 ppm (Young et al., 1976). The 1,3,7,8- and 2,3,7,8-TCDD impurities in Agent Orange have been reported to originate from 2,4,5-T synthesis (Buser, 1978).

Though 2,4-D is not expected to contain 2,3,7,8-TCDD and does not in general (Woolson et al., 1972; Henshaw et al., 1975), production trains are often used for production of chemicals whose manufacture necessitates the use of similar process equipment. In the manufacture of chemicals on a production train previously contaminated with PCDDs, both the products and waste generated can be contaminated with PCDDs. Thus, 2,4-D, which otherwise was not expected to be contaminated with 2,3,7,8-TCDD, did indeed contain some 2,3,7,8-TCDD because the equipment used in its manufacture had been employed previously to produce 2,4,5-T (45 FR 32677).

1.2.1.3. **DIPHENYL ETHERS** -- The presence of TCDDs, PeCDDs and HxCDDs as contaminants in diphenyl ether herbicides was reported by Yamagishi et al. (1981). The source of PCDDs in these herbicides was speculated to be reaction of the trichlorophenol used in their production. The contaminants could also be present in the trichlorophenol feed stock. The concentrations of the two major impurities, namely, TCDDs and PeCDDs, in commercial formulations were ~150 and 30 ppm, respectively. The isomeric distribution of TCDDs showed that the major components were 1,3,6,8- and 1,3,7,9-isomers. The 2,3,7,8-TCDD isomer was not detected in the commercial products. Lindahl et al. (1980) showed that 25% of chlorinated diphenyl ethers decomposed at 500°C, but over 99% at 700°C, with highest PCDD yields at 600°C. PCDD production occurred only from loss of chlorines in the ortho position.

1.2.1.4. **CHLOROBENZENES** -- Pyrolysis of tri-, tetra- and pentachlorobenzenes in sealed quartz ampoules produced small amounts of PCDDs in addition to PCDFs, chlorophenols and chloronaphthalenes (Buser, 1979; Rappe et al., 1983b).

1.2.1.5. **INCINERATION OF SELECTED INDUSTRIAL WASTES** -- The combustion of a variety of chlorinated hydrocarbons has been shown to produce PCDDs. The formation of PCDDs would likely occur in incinerators operating at 750-900°C; chlorophenols are probably the major precursors of PCDDs formation. At temperatures >1200-1400°C, PCDDs are likely to decompose under the residence times used in most incinerators (Junk and Richard, 1981; Redford et al., 1981; Shaub and Tsang, 1983).

1.2.2. **Municipal Incinerators.** PCDDs have been detected both in the fly ash and air particulate matter from municipal incinerators by several investigators in Canada, Europe and the United States (Gizzi et al., 1982; Benfenati et al., 1983; Taylor et al., 1983; Olie et al., 1982, 1983; Lustenhouwer et al., 1980; Barnes, 1983; Tiernan et al., 1982; Bumb et al., 1980). The particulate matter forming the emissions (air particulates) has been reported to have a 10-fold greater concentration of PCDDs than the precipitated material (fly ash) (NRCC, 1981a). The concentration of total

TCDDs, PeCDDs and HxCDDs in the fly ash from a variety of municipal incinerators in Canada, Europe and the United States varied from 3.2-110, 3.4-488 and 2.2-1200 μg/kg, respectively (NRCC, 1981a). In a comparative study of municipal fly ash conducted between a single incinerator in the United States and one in Europe, Lamparski and Nestrick (1980) detected at least 14 of the 22 possible TCDD isomers. Although the ratio of isomers to the total present were similar in both fly ashes, their absolute amounts varied by a factor $\geq 10$. The fly ash from this European incinerator always had higher individual isomeric concentrations than the fly ash from the United States incinerator. For example, the concentrations of 2,3,7,8-TCDD in the United States fly ash and this European fly ash were 0.43 and 23 μg/kg, respectively. Buser and Rappe (1980; 1983) have studied fly ash samples from municipal incinerators in Switzerland and Canada. Their results revealed the presence of a complex isomeric mixture with at least 17 TCDDs, 12 PeCDDs and 8 HeCDDs present.

1.2.3. **Other Combustion Processes.** Dow Chemical Co. (1978) reported the presence of PCDDs in particulate matter from most combustion sources. This led to their hypothesis that PCDDs may be formed in trace amounts from the chemical reactions of combustion of many chlorinated hydrocarbons (Bumb et al., 1980; Crummett et al., 1981). These investigators detected PCDDs including TCDD and HxCDD in particulate matter from municipal and industrial incinerators, from mufflers of diesel trucks and passenger vehicles, from home wood-burning fireplaces, from soot and from cigarette smoke. Since the "trace chemistries of fire" hypothesis was presented, several investigators have attempted to test it. Although there is general agreement regarding the production of PCDD from burning natural woods with additional HCl and from incinerators, their production from some combustion sources (such as gas burners and auto and truck engines) is not generally accepted (NRCC, 1981a).

**1.2.4. Miscellaneous Sources.** At present, other potential sources of PCDDs are chemicals and wastes known to be contaminated with PCDDs but withdrawn from use and awaiting disposal, and disposal sites where chemical wastes containing PCDDs have been dumped. In addition, Lamparski et al. (1980) reported that photolysis of pentachlorophenol-treated woods may be also lead to the formation of PCDD.

### 1.3. RELATIONSHIP BETWEEN SOURCES AND CONTAMINATION IN ENVIRONMENTAL MATRICES

The potential relationship between various sources of PCDDs and the environmental matrices where PCDDs have been detected (NRCC, 1981a) is depicted in Figure 1-1. Figure 1-1 has been modified to indicate the possible inhalation exposures from these sources.

### 1.4. SYNTHESIS

Not all of the 75 possible PCDDs have been synthesized in the laboratory, and most of the available synthetic methods are characterized by poor yields and chromatographic difficulties in producing pure isomers. This has contributed to the dearth of toxicological data on more than a few PCDDs.

The 2,3,7,8-TCDD isomer has received the most attention. There are five major synthetic methods (U.S. EPA, 1980a):

1. Reaction of dichlorocatechol salts with o-chlorobenzene by refluxing in alkaline dimethylsulfoxide solution (19% yield). A better method is the reaction of o-dichlorocatechol with dichloro-substituted o-chloronitrobenzene (Gray et al., 1976).

2. Chlorination of dibenzo-p-dioxin with chlorine in the presence of ferric chloride. Some penta- and tri-CDDs are also produced. If iodine is also utilized as an initiator in the presence of ferric chloride/chlorine, the yield is 44%.

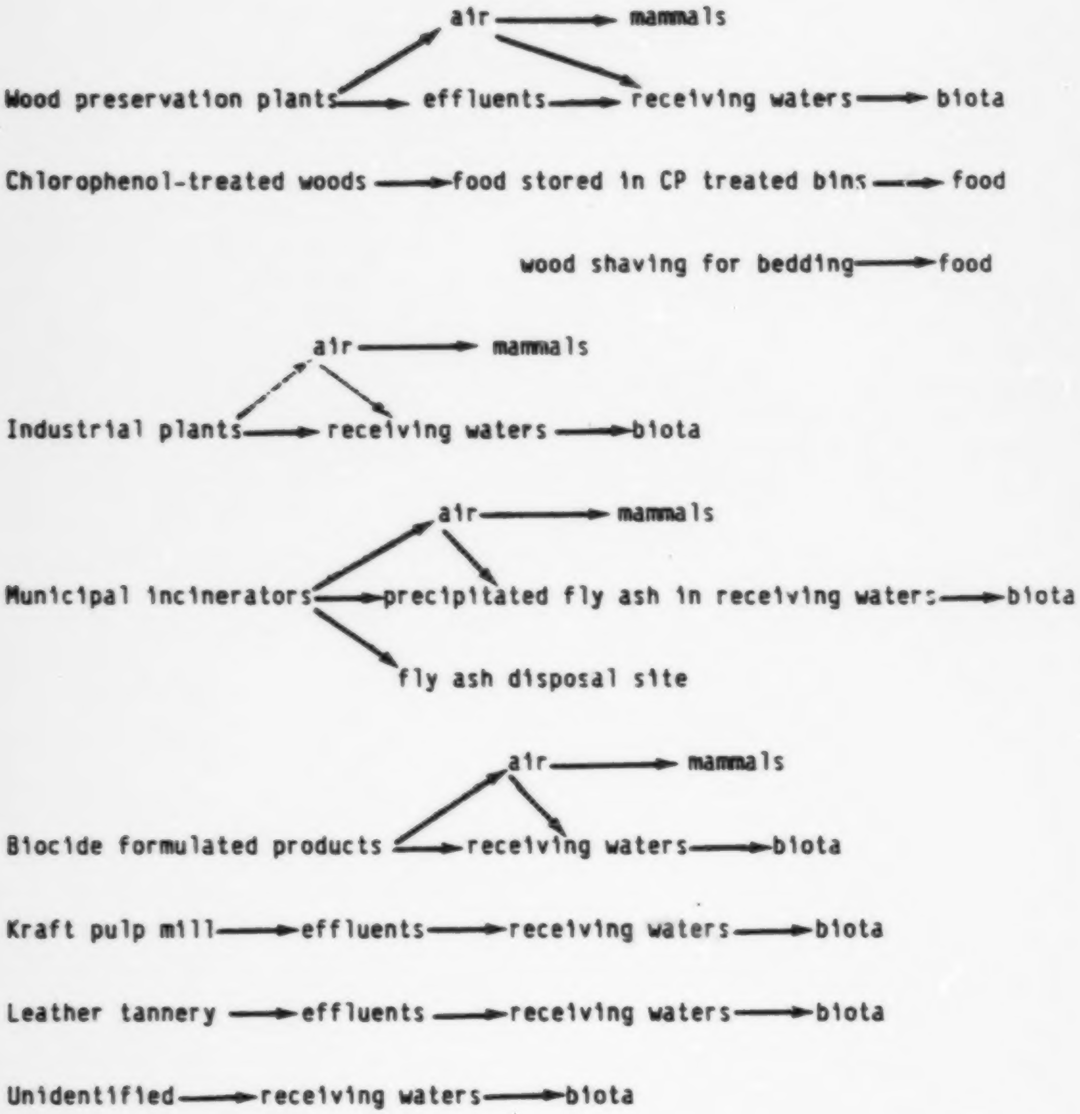

air
mammals
Wood preservation plants
effluents
receiving waters
biota
Chlorophenol-treated woods
food stored in CP treated bins
food
wood shaving for bedding
food
air
mammals
Industrial plants
receiving waters
biota
air
mammals
Municipal incinerators
precipitated fly ash in receiving waters
biota
fly ash disposal site
air
mammals
Biocide formulated products
receiving waters
biota
Kraft pulp mill
effluents
receiving waters
biota
Leather tannery
effluents
receiving waters
biota
Unidentified
receiving waters
biota

Figure 1-1

Possible potential relationship between various sources of PCDDs and the environmental matrices where PCDDs have been detected

Source: Modified from NRCC, 1981a

3. Irradiation by a mercury arc of mixtures of highly chlorinated dioxins in organic solvents allows production of a mixture of lower PCDDs, which have to be further chromatographed if pure isomers are desired. These are useful as gas chromatographic standards.

4. Self-condensation of identical chlorinated phenolates, or condensation of different chlorinated potassium phenolates (Ullman Reaction) at 180-400°C for 1-4 hours at pressures >1 atm. produces a complex mixture of PCDDs which requires further chromatography to isolate pure products. There are many competing reactions, some often encouraged by the catalysts utilized (e.g., copper, iron and aluminum salts or iodine).

5. Pyrolysis of chlorinated phenolates and of phenols. Though yields are low, this route is responsible for the presence of PCDD traces during manufacturing processes involving these starting compounds. This aspect has been covered in Section 1.2.1.1. Using this method, all 22 TCDDs, 14 PeCDDs and 10 HxCDDs have been synthesized (Buser and Rappe, 1980, 1983).

## 1.5. ANALTYICAL METHODS

The analytical methodologies for the separation of the isomeric PCDDs are difficult and expensive. Because of this, many investigators, particularly the earliest ones, did not characterize the individual isomers and it is not always clear whether a specific isomer or a mixture of isomers was responsible for the observed toxicological effect(s). Analytical methods are legion for the dioxins, particularly the cleanup procedures. Only the final step in separation/detection/quantitation will be considered in this section.

First, it must be appreciated that dioxins (and dibenzofurans) with halogens substituted in the 2,3,7,8-positions are far more toxic than dioxins without halogens in these positions. Furthermore, since octa-CDD is far less toxic, the presence of H atoms next to these positions is imperative for the PCDD to show greatest toxicity. Such isomer-specific detection requires analytical methods of great specificity and flexibility to handle the many different environmental and biological matrices involved. There are several techniques: radioimmunoassay, EC/GC, GC/MS, GC/SIM/MS, and miscellaneous techniques (Karasek and Onuska, 1982).

Radioimmunoassay is a promising experimental screening technique (Albro et al., 1979). The PCDD part of the sample is extracted, purified and then solubilized by sonication in a detergent/buffer mixture. Incubation with antiserum containing rabbit antibodies follows. After incubation, a $^{125}$I-labeled 2,3,7,8-TCDD derivative is then added to saturate the binding sites of the antibodies, and enough goat antiserum is added to precipitate completely the rabbit antibodies containing the bound PCDDs. After centrifugation and separation, the residue is measured for its gamma radiation. The decrease relative to a blank is proportional to the level of PCDDs in the sample. This technique is not isomer specific and will detect 10 pg/mℓ to 100 ng/mℓ of PCDDs, and is less costly than the methods considered next.

EC/GC using a packed or capillary column is another screening technique of somewhat general utility, attained by matching retention times. However, EC/GC responds to many compounds and high resolution MS confirmation is essential to identify the specific PCDDs. Hence, upper limit estimate levels are found for the sample, assuming recoveries are 100% and sampling has been representative. The method is rapid. Potentially, WCOT chromatography is capable of separating all 75 dioxin isomers, and the 22 TCDD, 14 PeCDD, and 10 HxCDD isomers have been resolved (Buser and Rappe, 1980, 1983), using a two or three column technique. The sensitivity ranges from 500 pg/mℓ to 100 ng/mℓ of ready to be injected sample.

Packed or capillary column GC/MS has been widely used to identify whether the GC separation has been specific (NRCC, 1981b). Since the PCDDs have intense molecular ions, the presence of the various PCDDs is easily confirmed by the characteristic isotope ratios of the peaks adjacent to the molecular ion. Isomer specificity is possible via the power of the preceding chromatographic separation and knowing the retention time. GC/MS is sensitive from 50 ng/mℓ to 15 µg/mℓ. However, use of the SIM mode of MS, wherein only the current carried by one (or more) diagnostic fragment ion(s) is measured, allows the sensitivity to reach 10 pg/mℓ. Diagnostic peaks other than the parent ion peak need to be noted to ensure specificity since the GC column may not resolve the compounds if a complex matrix is being investigated. In practice, detection limits from complex matrices (e.g., fly ash) are around 1 µg/mℓ. The advent of the isotopically labeled ${}^{37}Cl_4$ and ${}^{13}C_{12}$-2,3,7,8-TCDD internal standard isotope dilution technique evolved by Harless et al. (1980) allows a lower limit of 1.0±0.23 pg to be attained even in fly ash matrices via the SIM/MS methodology. This is equivalent to a 1 pg/µℓ or 1 ppb level in the sample before injection. The MS peaks used via SIM/MS are generally,

m/e: 327.8847 ($C_{12}H_4O_2{}^{7}Cl_4$), 321.8936 ($C_{12}H_4O_2{}^{5}Cl_3{}^{7}Cl$)

and 319.8965 ($C_{12}H_4O_2{}^{5}Cl_4$)

The typical concentrations of standards are: 50-300 ng/mℓ for the labeled compound and 1-5 ng/mℓ for unlabeled 2,3,7,8-TCDD. For highly noisy chromatograms or spectra, m/e 256.9327 and 258.9298 must be included (loss of Cl then CO from the molecular ion). MS resolution of better than 12,000-18,000 is often necessary to resolve interfering ions. At the moment, this method is the best available for pg analysis, although it is extremely costly. Levels as low as 100 fg have been detected by this method.

Other techniques are also available (e.g., chemical ionization MS, atmospheric ionization MS, negative ionization MS, and MS/MS) (NRCC, 1981b). The high cost of MS/MS has precluded general usage but pg levels have been detected for 2,3,7,8-TCDD (Chess and Gross, 1980). Nestrick et al. (1979) used reversed and normal phase HPLC to isolate PCDDs, followed by packed column GC/MS, an interesting instance of mixing chromatographic techniques before MS.

Analysts involved in collaborative laboratory studies for 2,3,7,8-TCDD are in general agreement at levels above 9 pg/mℓ of solution to be injected (Crummett, 1979). A greater concern is not so much the sensitivity of the end step, but rather the recovery of the cleanup procedures and the representativeness of the original sample, topics which are beyond the scope of this section.

## 1.6. SAMPLING AND CLEANUP

In general, dry samples are soxhlet extracted with benzene or toluene, while acid or alkaline hydrolysis of aqueous samples, including tissues, is followed by solvent extraction (e.g., toluene, benzene, hexane). Chloroform/methanol is effective for serum and plasma. Base hydrolysis at reflux destroys the more highly chlorinated dioxins, and a neutral extraction system is generally favored (Harless et al., 1980).

If lipids are present, these are partitioned by sulfuric acid treatment and subsequent extraction of the PCDDs in hexane (NRCC, 1981b). Column chromatography on acidic silica gel has also been used for this step. The next cleanup step usually involves column chromatography with alumina, Florisil, silica gel, or foam charcoal (Harless et al., 1980) to remove common chlorinated hydrocarbons (e.g., PCBs/pesticides). Further cleanup steps could involve HPLC (Nestrick et al., 1979), using reverse or normal phases before the final analytical step. In general, chlorinated dibenzofurans and chlorinated diphenyl ethers will also be included in the PCDD fractions.

It is important to determine recoveries for each environmental matrix. Soils of high organic content are difficult to analyze because absorption of PCDDs is much more extensive and hydrolysis steps or soxhlet extraction conditions have to be carefully optimized to allow intimate contact of the extracting solvent with the environmental matrix. Thus, the selection of adequate controls upon which to perform recovery experiments is very important. One would not, for example, select a soil of low organic content if the sample of interest happened to have a high organic content. Also, control soils should be selected from soils thought to be uncontaminated in the same area as the sample soil.

The past history of the sample is also important. Surface samples taken for soil analysis would contain low levels if frequently plowed soils were selected even if the analytical methods are adequate. Sampling of clear river water should generally contain lower levels of PCDDs than waters containing sediments (since PCDDs have a tendency to concentrate on particulate matters), or if a sample containing sediment were not shaken thoroughly.

Air samplers of Silvex or 2,4,5-T spraying will also contain low levels if the wind is variable. Sampling strategy can therefore predetermine PCDD levels before any cleanup or analytical steps.

## 2. ENVIRONMENTAL FATE AND TRANSPORT PROCESSES

### 2.1. WATER

**2.1.1. Biodegradation.** 2,3,7,8-TCDD exhibits relatively strong resistance to biodegradation. Only five of ~100 microbial strains that have the ability to degrade persistent pesticides show slight ability to degrade 2,3,7,8-TCDD (Matsumura and Benezet, 1973). Ward and Matsumura (1977) studied the biodegradation of $^{14}$C-labeled 2,3,7,8-TCDD in Wisconsin lake waters and sediments. The observed half-life of 2,3,7,8-TCDD in sediment-containing lake waters was found to be 550-590 days. In lake water alone, ~70% of the 2,3,7,8-TCDD remained after 589 days. It is highly likely that the apparent biodegradation loss was due to volatilization through air/water interface. Other investigators (Huetter and Phillip, 1982) have demonstrated the virtually complete lack of biodegradability of 2,3,7,8-TCDD under laboratory conditions. PeCDD and HxCDD, having more chlorine substitution on benzene rings, may be even more resistant to biodegradation than 2,3,7,8-TCDD.

**2.1.2. Phototransformation.** 2,3,7,8-TCDD has a UV absorption maximum at 310 nm with an extinction coefficient of 5590 $M^{-1}$ $cm^{-1}$ (NRCC, 1978). Solid 2,3,7,8-TCDD is photochemically stable but in the presence of a hydrogen donor [e.g., organic solvents, or when adsorbed on aluminum or silica gel (Liberti et al., 1978)], it will be photolyzed in sunlight (Crosby and Wong, 1977).

From the previous discussion of the photolysis of PCDDs in the presence of organic hydrogen donating organic solvents (see Section 1.1.), it is difficult to predict the photolytic fate of PCDDs in natural aquatic media where sufficient organic hydrogen donating substrate(s) may or may not be available. The situation is complicated further by the fact that the predominant amount of PCDDs in surface water may remain sorbed on suspended particles and sediments (Section 2.1.5.). Moreover, since the penetration of UV light into natural water may be limited, photolytic degradation of PCDDs in water may not often be of environmental importance. The influence of natural sensitizers on potential photodegradation has not been investigated.

2.1.3. **Radical Oxidation and Hydrolysis.** Hydrolysis of 2,3,7,8-TCDD or oxidation with free radicals ($RO_2\cdot$, $RO\cdot$, etc.) in aquatic media are not likely to be of environmental significance (Callahan et al., 1979; Mabey et al., 1981). Likewise, hydrolysis and oxidation are even less likely to be environmentally significant processes for PeCDD, and HxCDD (Mabey et al., 1981).

2.1.4. **Volatilization.** Although several investigators implicated volatilization as one of the major reasons for the observed disappearance of 2,3,7,8-TCDD from aqueous solution during microbial studies, no quantitative information regarding the volatilization of 2,3,7,8-TCDD from aquatic media is available (Ward and Matsumura, 1977; Huetter and Phillip, 1982). Using the formulae of Liss and Slater (1974), a vapor pressure value of $1.7\times10^{-6}$ torr and a solubility value of $6.2\times10^{-10}$ mole/ℓ, NRCC (1981a) calculated the volatilization half-life for 2,3,7,8-TCDD to be 6 minutes from turbulent water of 1 cm depth and 10 hours from water of 1 m depth. The limitations of this theory in predicting the rate of volatilization have been discussed in the NRCC (1981a) document. The Liss-Slater model does not consider terrestrial matrices (suspended solids, sediments, biota, etc.) normally encountered in natural surface water. Employing a computerized EXAMS modeling for two standardized (see NRCC, 1981a, for definitions) aquatic ecosystems (lake and pond) and the input parameters for 2,3,7,8-TCDD given in NRCC

(1981a), volatilization has been estimated to account for 100% of the fraction lost; biodegradation has been calculated to be 0%. The volatilization half-life for 2,3,7,8-TCDD has been estimated to be 5.5 years and 12 years from pond and lake water, respectively.

These wide variations in the calculated half-life are conceivable when examined with the mathematical form of volatilization model (Thibodeaux, 1979). Evaporation half-life is shown to be proportional to water depth and inversely proportional to the mass-transfer coefficients. The EXAMS calculation routine contains an added element that accounts for the sorption of 2,3,7,8-TCDD on both the suspended and the bottom sediment. For substances with high sorption coefficients (e.g., 2,3,7,8-TCDD), the evaporation rate is reduced significantly. A comparision of calculated transport rates of 2,3,7,8-TCDD from an industrial site indicates that the evaporation half-life from a contaminated cooling water pond sediment is negligible in comparison to other contaminated areas on the site (Thibodeaux, 1983). Information regarding the volatilization of PeCDDs and HxCDDs from aquatic media is not available. These compounds, however, with higher molecular weight and more chlorine subsitution, are expected to volatilize more slowly than 2,3,7,8-TCDD from aquatic media.

2.1.5. Sorption. Data from microcosm experiments indicate that 2,3,7,8-TCDD is highly sorbed to sediments and biota (Isensee and Jones, 1975; Ward and Matsumura, 1977). More than 90% of 2,3,7,8-TCDD in an aquatic medium may be present in the adsorbed state (Ward and Matsumura, 1977). Considering the low water solubility and the expected high octanol/water partition coefficient, this is not surprising. In fact, the equation of Karickhoff et al. (1979) predicts a sorption partition coefficient value of $10^4$ for 2,3,7,8-TCDD in sediments containing 2% organic carbon (NRCC, 1981a).

Similarly, the higher PCDDs are likely to be present predominantly in the sediment-sorbed state in aquatic media.

## 2.2. AIR

A number of PCDDs, including TCDDs, PeCDDs and HxCDDs, have been detected in the dust and fly ash from municipal incinerators (Cavallaro et al., 1980; Clement and Karasek, 1982; Eiceman et al., 1981). Size fractionations of fly ash from municipal incinerators have shown that larger concentrations of TCDDs and PeCDDs occurred on the larger (550 μm) particles, while the 30 μm particles had greater relative concentrations of octa-CDD (Clement and Karasek, 1982). Therefore, PCDDs emitted to the atmosphere from combustion processes appear to be associated with air particulate matter (Nestrick et al., 1980). Cupitt (1980) presented a mathematical description of a physical removal mechanism for the determination of the fate of toxic and hazardous materials in the air environment. For the absorption of chemicals on aerosol particles, he developed a general model relating the sorption on aerosol surface area and the saturated vapor pressure of a chemical. His results suggests that absorption will be a reasonable vapor-phase removal mechanism from air only for materials with saturation vapor pressure of $10^{-7}$ torr or less. 2,3,7,8-TCDD has a vapor pressure of $1.7\times10^{-6}$ torr.

Photodegradation and wet and dry deposition of particulate-bound PCDDs are probably the most important rate-determining processes for the atmospheric PCDDs. The available data relating the photodegradation of PCDDs in the sorbed phase or as films are conflicting. For example, experiments of earlier investigators involving photoreactivity of 2,3,7,8-TCDD as films or sorbed on solid surfaces and exposed to the atmosphere yielded negligible photodegradation with sunlight (Crosby et al., 1971). The results of Buser (1978) and Buser and Rappe (1978) have shown that higher PCDDs may undergo photolytic reductive dechlorination under mercury arc UV radiation.

Getefuegi et al. (1977) studied 2,3,7,8-TCDD photochemical degradation under simulated environmental conditions by exposing silica gel-sorbed 2,3,7,8-TCDD to light of wavelength >290 nm and observed 92% decomposition in 7 days. Silica gel is a hydrogen donor and so facilitates the decomposition. The half-life for photodegradation of 2,3,7,8-TCDD film on glass surfaces has been estimated to be 5.8 days under irradiation with sunlamps (Nestrick et al., 1980). It is not known whether a similar photodegradation of particle-bound 2,3,7,8-TCDD will occur in the atmosphere since the state of sorption may be different from those obtained under laboratory conditions. Based on the results of photolysis of particle-bound polynuclear aromatic hydrocarbons, some of which substantially photodegrade in solution (Santodonato et al., 1981), it is unlikely that substantial photodegradation of PCDD will occur in the atmosphere. The potential for oxidation of PCDDs by free radicals (OH•, O•, etc.) and other molecules ($O_3$, $NO_x$, etc.) that may be present in the atmosphere is not known.

## 2.3. SOIL

**2.3.1. Sorption.** From the empirical correlation equation developed by Karickhoff et al. (1979) it is possible to predict a soil/water partition coefficient of $4.8 \times 10^4$ for a soil containing 10% organic matter (NRCC, 1981a).

Because of their high affinity toward soils, particularly those with significant organic content, and because of their extremely low water solubilities, 2,3,7,8-TCDD (and presumably other PCDDs) tend to remain on or near the surface of soils (U.S. EPA, 1984). With time, 2,3,7,8-TCDD bound to soil becomes more difficult to desorb (Phillip et al., 1981; Huetter and Phillip, 1982). Several authors have shown that vertical movement of 2,3,7,8-TCDD in soil is negligible (U.S. EPA, 1980a). Movement of

2,3,7,8-TCDD does occur via horizontal transfer (by water transport) and through contaminated airborne dust particles (U.S. EPA, 1984). Therefore, underground water supplies are unlikely to be contaminated with 2,3,7,8-TCDD unless the water carries a large particulate concentration of high organic content. As the organic content of soil decreases or in situations where soil binding sites are saturated, however, the likelihood of vertical movement of PCDDs in soil increases. In areas of heavy rainfall and sandy soil, vertical migration of 2,3,7,8-TCDD and its lateral displacement via soil erosion and runoff would be enhanced (U.S. EPA, 1984). The migration of 2,3,7,8-TCDD far beyond the surface layer of soil has been reported around Seveso, Italy (Cavallaro et al., 1979). It was suggested that this was due to the presence of fissures in soil and abnormally high rainfall. In view of the low water solubility of 2,3,7,8-TCDD, another explanation of this extensive migration could be due to saturation of sorption sites in soil or solvation of 2,3,7,8-TCDD by organic solvents (NRCC, 1981a). Alternatively, the PCDDs could have been carried deeper by the soil particles themselves. It it therefore, possible that 2,3,7,8-TCDD may appear in normal water leachate of soils, particularly after multiple PCDD application or accidental release of 2,3,7,8-TCDD on soil. Results of off-site transport calculations from contaminated soil surfaces are available (Thibodeaux, 1983). These calculations show that between 120-1200 g/year of 2,3,7,8-TCDD volatilized from a highly contaminated soil surface between 1978 and 1979 prior to the implementation of remedial measures. Over the same period it was estimated that 28-37 g/year left the site by wind-blown entrainment, 0.1-1.0 g/year evaporated from a burial site, and 0.98-2.3 g/year were in runoff water. All these sources were areas in which the 2,3,7,8-TCDD was sorbed in soil (Thibodeaux, 1983).

2.3.2. **Phototransformation.** Kearney et al. (1972) indicated that pure 2,3,7,8-TCDD on soil surfaces was not degraded by sunlight, but a formulation of herbicide containing 2,3,7,8-TCDD significantly degraded (~15% in 6 hours) on soil surface by the action of sunlight. The herbicide formulation provided a hydrogen donor that probably allowed the photolysis to occur. A similar result was reported by Crosby and Wong (1977). Following application of Agent Orange containing 15 ppm 2,3,7,8-TCDD to soil as a film of 10 mg/cm$^2$ thickness and irradiation with natural sunlight, about 10% of 2,3,7,8-TCDD photodegraded after 6 hours. The photodegradation, was however, complete in 6 hours after application to leaf surfaces. The authors attributed the slower photolysis of 2,3,7,8-TCDD in soil to a shading effect by lower layers of soil particles.

2.3.3. **Biodegradation.** The biodegradation of 2,3,7,8-TCDD in soil is probably faster than in surface water (Young et al., 1976). The biodegradation rate will also be fastest in soils of high organic content. It can, however, be concluded from the following discussions that the biodegradation half-life in soil is likely to be more than 1 year.

The overall half-life of 2,3,7,8-TCDD in soil has been reported to be 1-3 years by Kearney et al. (1972) and 3 years by Young et al. (1976). The half-life of 2,3,7,8-TCDD following an accidental 2,3,7,8-TCDD release from a trichlorophenol manufacturing plant at Seveso, Italy, was studied by DiDomenico et al. (1980). At the time of the release these investigators estimated the half-life of 2,3,7,8-TCDD in soil to be 5 months. One month after release, the 2,3,7,8-TCDD half-life was estimated to be 1 year, whereas 17 months later it was estimated to be >10 years. These may greatly underestimate the true value, however, since it has recently been shown that radiolabeled TCDD adsorbed to soil becomes progressively more resistant to extraction (Phillip et al., 1981; Huetter and Phillip, 1982). More recent data (Young, 1983; Wipf and Schmid, 1983) indicate that the half-life is closer to 10 years. Since most of the other PCDDs are no more susceptible to microbial degradation than TCDDs, their half-lives in soil are presumed to be similar to that postulated for TCDDs.

## 2.4. FOOD

Studies with either the seeds or the mature plants of soybeans or oats showed that 2,3,7,8-TCDD was neither absorbed by the seeds after spraying nor taken up from the soil into the mature plants (U.S. EPA, 1984). Cocucci et al. (1979), however, reported 2,3,7,8-TCDD in leaves, twigs, fruits and cork of several woody plants growing in soil that had been contaminated by the release of 2,3,7,8-TCDD in Seveso, Italy.

A bioconcentration factor (BCF) relates the concentration of a chemical in aquatic species to the concentration in water. Several equations have been developed for predicting the steady-state BCF for an organic compound from its octanol-water partition coefficient (Kenaga and Goring, 1980; Veith et al., 1980; Veith and Kosian, 1983). All of these depend on the availability of useful value for the partition coefficient. Several estimated values (Leo, 1979; Mabey et al., 1981; Neely, 1983) and one measured value (Neely, 1979; Kenaga, 1980; Neely, 1983) have been reported for the octanol-water partition coefficient for 2,3,7,8-TCDD. Use of six equations with four values for the partition coefficient, $K_{ow}$, results in the following predicted BCFs (Table 2-1). The predicted BCFs range from 7000-900,000 using the calculated values of the partition coefficient and from 3000-68,000 using the one measured value.

Several measured BCFs have been reported for 2,3,7,8-TCDD (Table 2-2), but none can be considered definitive values. Many were determined in model

## TABLE 2-1

## Predicted BCFs from Calculated and Measured Values of $K_{ow}$*

| Equation | log $K_{ow}$ | | | |
|---|---|---|---|---|
| | Calculated | | | Measured |
| | 6.84 | 7.14 | 7.28 | 6.15 |
| log BCF = 0.542 log $K_{ow}$ + 0.124 | 6,780 | 9,860 | 11,700 | 2,870 |
| log BCF = 0.76 log $K_{ow}$ - 0.23 | 93,000 | 157,000 | 201,000 | 27,800 |
| log BCF = 0.79 log $K_{ow}$ - 0.40 | 101,000 | 174,000 | 224,000 | 28,740 |
| log BCF = 0.635 log $K_{ow}$ + 0.7285 | 118,000 | 183,000 | 225,800 | 43,000 |
| log BCF = 0.85 log $K_{ow}$ - 0.70 | 130,000 | 234,000 | 308,000 | 33,700 |
| BCF = 0.048 $K_{ow}$ | 332,000 | 663,000 | 915,000 | 67,800 |

*Source: U.S. EPA, 1983a

## TABLE 2-2

## Bioaccumulation of 2,3,7,8-TCDD in Freshwater Species by Aquatic Organisms[a]

| Species | Tissue | Duration (days) | Bioconcentration Factor | Reference |
|---|---|---|---|---|
| Alga,<br>*Oedogonium* *cardiacum* | NS | 33 | 2634 | Isensee, 1978 |
| Alga,<br>*Oedogonium* *cardiacum* | NS | 32 | 2075<br>2083 | Isensee, 1978<br>Yockim et al., 1978 |
| Snail,<br>*Physa* sp. | whole body | 33 | 3772 | Isensee, 1978 |
| Snail,<br>*Physa* sp. | whole body | 32 | 3775<br>3731 | Isensee, 1978<br>Yockim et al., 1978 |
| Cladoceran,<br>*Daphnia* *magna* | whole body | 30 | 3398 | Isensee, 1978 |
| Cladoceran,<br>*Daphnia* *magna* | whole body | 32 | 7070<br>7125 | Isensee, 1978<br>Yockim et al., 1978 |
| Catfish,<br>*Ictalurus* *punctatus* | whole body | 28 | 2000 | U.S. EPA, 1983b;<br>Thomas, 1983 |
| Mosquitofish,<br>*Gambusia* *affinis* | whole body | 14 | 4850<br>4875 | Isensee, 1978<br>Yockim et al., 1978 |
| Rainbow trout,<br>*Salmo* *gairdneri* | NS | NS | 9080[b] | Neely, 1979 |
| NS | NS | NS | 5400 | Kenaga, 1980 |

[a] Source: U.S. EPA, 1983a

[b] Calculated as ratio of uptake and clearance rate constants

NS = Not specified

ecosystems in which the concentrations in water were not necessarily constant. The measured BCFs, however, range from 390-13,000. Branson et al. (1983) in a poster presentation at a recent meeting reported laboratory experimentation data on uptake and depuration rate constants of $^{14}C$ 2,3,7,8-TCDD in rainbow trout to be 108±3 mℓ water/g whole fish/day and 0.012±0.001 days, respectively. The fish were exposed to 107 ng/ℓ for 6 hours of $^{14}C$ 2,3,7,8-TCDD in static water followed by a flowthrough depuration period of 139 days. BCF was determined to be 9270±1050. At the same meeting Millner et al. (1983) reported, in a poster presentation, that calculated BCF values for 2,3,7,8-TCDD varied between 41,250 and 468,750. These BCF values were calculated from equilibrium constant data for partitioning of 2,3,7,8-TCDD between water and sediment reported by Isensee and Jones (1975). Until further information is available, the U.S. EPA's best current estimate for the BCF of 2,3,7,8-TCDD in aquatic organisms is 5000 (U.S. EPA, 1984). Uptake by organisms of 2,3,7,8-TCDD from lower trophic levels may add to uptake from water, so this BCF may underestimate concentrations in wild aquatic organisms. Corbet et al. (1983) conducted bioconcentration tests on radiolabeled 1,3,6,8-TCDD with the fathead minnow and the rainbow trout, using a 4-day uptake phase and a 48-day depuration phase. Based on radioactivity measurements, the projected steady-state BCFs were 1061 with the fathead minnow and 469 with the rainbow trout.

No bioconcentration studies have been conducted with any other PCDDs. The predicted BCFs for PeCDDs and HxCDDs should be higher than similarly predicted BCFs for 2,3,7,8-TCDD since the higher degree of chlorination would be expected to result in higher values of $K_{ow}$ (NRCC, 1981a).

However, since the available measured values of BCF for 2,3,7,8-TCDD are substantially lower than most of the predicted values, BCFs for PeCDDs and the HxCDDs probably cannot be accurately estimates from physical properties or structure alone.

# 3. EXPOSURE

Much of this section is taken directly from U.S. EPA (1984); additional information has been obtained from computer searches of more recent literature.

## 3.1. WATER

For 2,3,7,8-TCDD, the amount of human exposure that can be attributed directly to drinking water alone cannot be determined readily. The solubility of 2,3,7,8-TCDD in water is 0.2 ppb (Crummett and Stehl, 1973). According to a 1977 report by the National Academy of Sciences (NAS, 1977), no 2,3,7,8-TCDD had been detected in drinking water using methods with limits of detection in the ng/ℓ (ppt) range. PCDDs may occur in water containing suspended particulates. Particles tend to settle quickly to the bottom of a container, however, and the dioxins may not be detected unless the sample is well shaken, or unless the dioxin content of the particulate matter is analyzed separately.

## 3.2. AIR

No pertinent data regarding inhalation exposure to 2,3,7,8-TCDD were located in the available literature. It is clear that the spraying of 2,4,5-T will lead to a concomitant exposure to 2,3,7,8-TCDD, but it is not possible to estimate a typical exposure because of spray drift to nontarget sites, and because of the intermittent exposure during spraying. Nash and Beal (1978) reported detecting femtogram ($10^{-15}$ g) quantities of dioxin in the air following the application to microagroecosystems of either granular or emulsified Silvex formulations containing tritiated 2,3,7,8-TCDD. Airborne vapor-phase and particulate matter may represent a significant transport mechanism for dioxin in the environment. Several studies have shown the presence of dioxins in fly ash from municipal incinerators (Nilsson et al., 1974; Olie et al., 1977; Buser and Rappe, 1978; Tiernan and Taylor, 1980).

Recently, Eicemann et al. (1980, 1981) have shown that the concentrations of total TCDD, PeCDD and HxCDD isomers in fly ash from municipal incinerators in Ontario, Canada, range from 8.6-27, 3.4-45 and 2.2-53 ng/g fly ash, respectively. Another report contends that particulates from various combustion sources may contain significant amounts of PCDDs (Dow, 1978).

## 3.3. FOOD

The occurrence of 2,3,7,8-TCDD in food could result from spraying of some plant crops with 2,3,7,8-TCDD-contaminated herbicides, consumption by livestock of 2,3,7,8-TCDD-contaminated forage, or magnification of residues through the food chain. Conceivably, 2,3,7,8-TCDD could be deposited on food crops during the combustion of 2,4,5-T treated vegetation as originally suggested by Buu-Hoi et al. (1971). Galston (1979) discussed conditions under which 2,3,7,8-TCDD might be absorbed by humans from a 2,4,5-T-treated food chain and might accumulate in the fatty tissues and secretions, including milk.

Results of a collaborative beef monitoring program (U.S. EPA, Dow Chemical Company, Wright State University and Harvard University) showed that some 2,3,7,8-TCDD could be detected in the adipose tissue of cattle with access to 2,4,5-T-treated rangeland (U.S. EPA, 1984). Of the 85 beef fat samples analyzed, one sample contained 60 ppt of 2,3,7,8-TCDD and two samples appeared to have 2,3,7,8-TCDD levels in the range of 5-10 ppt. While several laboratories detected levels in this range, the values reported were very near the limits of detection.

2,3,7,8-TCDD has been reported in fish from Vietnam in 1970 (Baughman and Meselson, 1973) where levels up to 800 ppt were detected. Unspecified isomers of tetra-, penta-, hexa- and octa-CDD at total PCDD levels above 500 ppt were found in fish from the Tittabawasee River at the Dow dam, and Emerson Park sites in Midland, Michigan (Kuehl, 1981).

2,3,7,8-TCDD has been reported in several species of commercial and noncommercial fish in several rivers and lakes in the United States and Canada. The levels of 2,3,7,8-TCDD in fish and shellfish as determined by various authors are given in Table 3-1. Thus, fish may be a major source of PCDD exposure to the general public.

Results of analyses, shown in Table 3-1, indicate that the 2,3,7,8-TCDD levels in fish and shellfish depend not only on the sites from which they are collected, but also on the type of species collected. For example, fish and shellfish collected from Bayon Meto/Arkansas River, Titabawassee/Saginaw River, Saginaw Bay, Lake Ontario, Lake Huron, and Cayuga Creek showed higher levels of 2,3,7,8-TCDD than those collected from Lake Erie, Lake Michigan, Lake Superior and the Atlantic Ocean. In addition, certain types of aquatic species that are bottom feeders, have high fat content or are carnivorous (such as bottom feeders, catfish, carp, trout and salmon) showed higher levels of 2,3,7,8-TCDD than bass, bullhead or suckers. Cordle (1983) estimated the total human daily intake of 2,3,7,8-TCDD from consumption of fish. Assuming that fish is consumed in the amount of the 99th percentile (i.e., 36.8 g/day with an average 2,3,7,8-TCDD residue level of 25 ppt), he estimated the total daily intake of 0.92 ng for the 99th percentile population. Assuming an average body weight of 70 kg, this value corresponds to a total dietary intake of 13 pg/kg bw/day.

TABLE 3-1

Levels of 2,3,7,8-TCDDs in Fish and Shellfish

| Type/Section of Fish | Sampling Site | Concentration[a] (ppt) | Reference |
|---|---|---|---|
| Edible flesh[b] | Bayou Meto/Arkansas River | 480 | Mitchum et al., 1980 |
| Catfish | Bayou Meto/Arkansas River | ND (7)-50 | Mitchum et al., 1980 |
| Buffalo | Bayou Meto/Arkansas River | ND (7-13) | Mitchum et al., 1980 |
| Bottom feeder | Bayou Meto/Arkansas River | 77 | Mitchum et al., 1980 |
| Whole body | Tone River, Japan | 200 | Yamagishi et al., 1981 |
| Rock bass | Lake Ontario/Lake Erie/ Welland Canal | ND (<2) | Josephson, 1983 |
| Eel, smelt and catfish | Lake Ontario/Lake Erie/ Welland Canal | 2-39 | Josephson, 1983 |
| Crayfish | Bergholtz Creek, Love Canal | 3.7 | Smith et al., 1983b |
| Catfish, bass and walleyed pike | 2,4,5-T contaminated watershed in Arkansas and Texas; Tittabawassee and Saginaw Rivers | ND (5-10) | Shadoff et al., 1977;<br>U.S. EPA, 1980a;<br>Buser and Rappe, 1980 |
| Lake trout/whole body | Lake Ontario | 51-107 | O'Keefe et al., 1983 |
| Chinook salmon/skinless fillet | Lake Ontario | 26-39 | O'Keefe et al., 1983 |
| Coho salmon/skinless fillet | Lake Ontario | 20-26 | O'Keefe et al., 1983 |

TABLE 3-1 (cont.)

| Type/Section of Fish | Sampling Site | Concentration[a] (ppt) | Reference |
|---|---|---|---|
| Rainbow trout/skinless fillet | Lake Ontario | 17-32 | O'Keefe et al., 1983 |
| Brown trout/skinless fillet | Lake Ontario | 8-162 | O'Keefe et al., 1983 |
| White perch/skinless fillet | Lake Ontario | 17-26 | O'Keefe et al., 1983 |
| White sucker/skinless fillet | Lake Ontario | ND (3.2)-10 | O'Keefe et al., 1983 |
| Smallmouth bass/skinless fillet | Lake Ontario | 5.9 | O'Keefe et al., 1983 |
| Brown bullhead/skinless fillet | Lake Ontario | 3.6 | O'Keefe et al., 1983 |
| Carp/Goldfish/skinless fillet | Cayuga Creek | 87 | O'Keefe et al., 1983 |
| Northern pike/skinless fillet | Cayuga Creek | 32 | O'Keefe et al., 1983 |
| Pumpkin seed/skinless fillet | Cayuga Creek | 31 | O'Keefe et al., 1983 |
| Rock bass/skinless fillet | Cayuga Creek | 12 | O'Keefe et al., 1983 |
| Coho salmon/skinless fillet | Lake Erie | 1.4-<3.5 | O'Keefe et al., 1983 |
| Walleye pike/skinless fillet | Lake Erie | 2.6 | O'Keefe et al., 1983 |
| Smallmouth bass/skinless fillet | Lake Erie | 1.6-<2.4 | O'Keefe et al., 1983 |
| Carp/goldfish/skinless fillet | Lake Erie | ND (2.6) | O'Keefe et al., 1983 |
| Lake trout/whole body | Lake Huron | 21 | O'Keefe et al., 1983 |
| Carp/skinless fillet | Lake Huron | 26 | O'Keefe et al., 1983 |

TABLE 3-1 (cont.)

| Type/Section of Fish | Sampling Site | Concentration[a] (ppt) | Reference |
|---|---|---|---|
| Channel Catfish/skinless fillet | Lake Huron | 20 | O'Keefe et al., 1983 |
| Sucker/skinless fillet | Lake Huron | 25 | O'Keefe et al., 1983 |
| Yellow Perch/skinless fillet | Lake Huron | ND (8.7) | O'Keefe et al., 1983 |
| Coho salmon/skinless fillet | Lake Michigan | ND (3.8) | O'Keefe et al., 1983 |
| Rainbow trout/skinless fillet | Lake Superior | 1.0 | O'Keefe et al., 1983 |
| Perch/sucker | Saginaw Bay | ND (3.8)-25 | Niemann et al., 1983[c] |
| Catfish | Saginaw Bay | 14-37 | Niemann et al., 1983[c] |
| Carp | Saginaw Bay | 23-47 | Niemann et al., 1983[c] |
| Catfish | Bayon Meto/Arkansas River | ND (3.8) | Niemann et al., 1983[c] |
| Bottom feeders[b] | Bayon Meto/Arkansas River | ND (6.7)-12 | Niemann et al., 1983[c] |
| Lake trout | Lake Ontario | 34-54 | Niemann et al., 1983[c] |
| Rainbow trout | Lake Ontario | 43 | Niemann et al., 1983[c] |
| Ocean haddock | Atlantic Ocean | ND (4.6) | Niemann et al., 1983[c] |
| Carp | Lake Huron | 3-28 | Stalling et al., 1983 |
| Channel catfish | Tittabawassee River, Saginaw River and Grand River | 28-695 | Harless and Lewis, 1982 |

## TABLE 3-1 (cont.)

| Type/Section of Fish | Sampling Site | Concentration[a] (ppt) | Reference |
|---|---|---|---|
| Carp | Tittabawassee River, Saginaw River and Grand River | ND (7)-153 | Harless and Lewis, 1982 |
| Yellow perch | Tittabawassee River and Saginaw River | ND (5)-20 | Harless and Lewis, 1982 |
| Small mouth bass | Grand River | 7-8 | Harless and Lewis, 1982 |
| Sucker | Tittabawassee River and Saginaw Bay | ND (4)-21 | Harless and Lewis, 1982 |
| Lake trout | Lake Michigan | ND (5) | Harless and Lewis, 1982 |
| Trout | Lake Ontario at Burlington, Canada | 61.2 | Ryan et al., 1983 |
| Trout | Lake Ontario at Toronto Harbor, Canada | 32.3 | Ryan et al., 1983 |
| Trout | Lake Huron at Burnt Island, Canada | 30.4 | Ryan et al., 1983 |

[a] When not detected, the detection limit is indicated within the parentheses.

[b] Organisms not further identified in the report.

[c] Only the GC/MS results of these authors are included in tabulation

[d] These are the mean concentrations in samples showing detectable levels of 2,3,7,8-TCDD.

ND = Not detected

The results of analysis for 2,3,7,8-TCDD and HxCDD in human milk samples were reported by Langhorst and Shadoff (1980). Six of nine samples analyzed showed 2,3,7,8-TCDD at levels slightly higher than the detection limits (0.2-0.7 ppt). All nine samples showed HxCDD at levels slightly higher than the detection limit (0.2-0.5 ppt). These results remain unconfirmed, however, because of the lack of precise and accurate data. Samples of some quarantined bovine milk from the State of Michigan showed no detectable levels of HxCDD at the detection limits of 25 ppt (Lamparski et al., 1978).

## 3.4. DERMAL

Dermal exposure is likely to be significant during the spraying of 2,4,5-T using Agent Orange formulations utilized before the ban on 2,4,5-T. However, accurate determinations of the amounts of 2,3,7,8-TCDD absorbed during such operations appear to be lacking.

## 4. PHARMACOKINETICS

Pharmacokinetic data were only available for 2,3,7,8-TCDD, and have been reviewed by Neal et al. (1982) and Manara et al. (1982). Pertinent data regarding the absorption, distribution, metabolism or excretion of PeCDDs or HxCDDs were not located in the available literature; however, the similarity in physical and chemical characteristics makes similarity in absorption and distribution possible.

### 4.1. ABSORPTION

Rose et al. (1976) demonstrated that 83% of a single oral dose of 1 μg/kg bw of radiolabeled 2,3,7,8-TCDD was absorbed by Sprague-Dawley rats. A similar degree of absorption also was observed following repeated oral doses. Poiger and Schlatter (1980) examined the oral and dermal absorption of 2,3,7,8-TCDD administered with different vehicles to rats. As an indicator of absorption, the levels of 2,3,7,8-TCDD were measured in the livers 24 hours after treatment. In the oral study, more 2,3,7,8-TCDD was found in the liver when administered in 50% ethanol (36%) as compared to vehicles containing suspensions of soil (24-16% depending on the increasing lengths of contact time with the soil prior to administration) or activated carbon (<0.07%). In the hamster, 73.5% of an oral dose was absorbed (Olson et al., 1980). Dermal absorption was also affected by the vehicle with lipophilic ointment and vehicles containing soil and activated carbon inhibiting absorption, while absorption (~14% of administered dose) occurred from hydrophobic ointments or methanol (Poiger and Schlatter, 1980).

### 4.2. DISTRIBUTION

2,3,7,8-TCDD is a lipophilic compound which is predominantly distributed to the liver and adipose tissue. Piper et al. (1973) detected in rats 3.18

and 2.60% of a single oral dose of 2,3,7,8-TCDD/g of liver and fat, respectively, 3 days after treatment. The majority (53%) of the administered dose was eliminated in the feces. Similar results were observed by Rose et al. (1976) 22 days after rats received a single oral dose of 1 μg of 2,3,7,8-TCDD. In this study, the liver and fat retained 1.26 and 1.25%/g tissue, respectively. Follwing repeated exposure, however, the liver contained five times as much 2,3,7,8-TCDD as the adipose tissue. In a comparison study of the distribution of a single oral dose of 2,3,7,8-TCDD in rhesus monkeys and rats, the liver of the monkeys retained 10% of the administered dose, while 40% was retained in the liver of the rats (Van Miller et al., 1976). Liver and adipose tissue were also the sites of disposition of 2,3,7,8-TCDD in rats maintained for 2 years on diets that provided 2,3,7,8-TCDD in doses of 0.1, 0.01 and 0.001 μg/kg bw/day (Kociba et al., 1978). The liver to adipose tissue ratio of 2,3,7,8-TCDD was 3:1 for the two high dose groups and 1:1 for the low dose group. It was estimated that steady state was reached within <13 weeks of maintenance on the 2,3,7,8-TCDD diet.

There is some evidence of sex differences in tissue distribution in rats. During 42 days of administration of 2,3,7,8-TCDD at 7 or 20 ppb in the diet, ~85% of the total body residue of male rats was located in the liver, while 70% of the total body residue of females was located in the liver (Fries and Marrow, 1975).

The ability of mouse liver to sequester 2,3,7,8-TCDD increases with prolonged exposure (Teitelbaum and Poland, 1978). The hepatic uptake of [$^3$H]2,3,7,8-TCDD in Swiss-Webster mice was maximal 12 hours after intraperitoneal injection. Hepatic uptake, expressed as percent of total dose, increased from 11.7% in control mice to 60.9% in mice that had been pretreated with a single dose of unlabeled 2,3,7,8-TCDD 36 hours previously.

This observation is consistent with other data, which indicate that 2,3,7,8-TCDD is a potent inducer of hepatic mixed-function oxidase and that >90% of the hepatic 2,3,7,8-TCDD is localized in the microsomes (Allen et al., 1975). The toxicity of 2,3,7,8-TCDD has been demonstrated to correlate with the affinity of the receptor which controls this induction in mice (Poland and Glover, 1980).

Olson et al. (1980) treated hamsters, the species least sensitive to 2,3,7,8-TCDD toxicity, with 650 μg/kg of labeled 2,3,7,8-TCDD by single intraperitoneal or gavage injections. In animals killed at 1, 3, 10 and 20 days after treatment, the label was predominantly in the liver, followed by adipose tissue and adrenals. Analysis of the liver indicated that the label was associated with unmetabolized 2,3,7,8-TCDD. In this study, nearly identical distribution patterns were observed regardless of the route of administration.

Distribution studies have found that muscle and skin of guinea pigs and monkeys also accumulate 2,3,7,8-TCDD (Gasiewicz and Neal, 1979; Nolan et al., 1979). In the infant monkey, these organs account for 55% of the administered dose (Van Miller et al., 1976). Conversely, as compared with other species, less 2,3,7,8-TCDD is distributed to the liver and adipose tissue of monkeys.

A number of investigations have been performed to determine whether 2,3,7,8-TCDD distributes to the developing fetus. Following a single oral dose of [$^{14}$C]2,3,7,8-TCDD to pregnant Fischer 344 rats, low levels of radioactivity were detected evenly distributed in the fetal tissues at days 14 and 18 of gestation (Moore et al., 1976). By day 21 of gestation, however, the radioactivity was detected at higher levels in the fetal liver as compared with other tissues. Nau et al. (1982) observed a sharp decrease in the uptake of 2,3,7,8-TCDD by fetal NMRI mice between days 10 and 11 of gestation, which coincided with placentation. As observed in rats, 2,3,7,8-TCDD had less affinity for fetal liver as compared with maternal liver in mice. Appelgren et al. (1983), using autoradiography, found the highest concentrations of radioactivity in the liver, followed by the nasal mucosa of fetal mice on day 17 of gestation after intravenous administration of [$^{14}$C]-2,3,7,8-TCDD to pregnant mice.

## 4.3. METABOLISM

Available information regarding the metabolism of 2,3,7,8-TCDD was reviewed recently by Neal et al. (1982). Piper et al. (1973) suggested that 2,3,7,8-TCDD must undergo at least some metabolism since a small portion of the radioactivity of a single oral radiolabeled dose of 2,3,7,8-TCDD was excreted in the urine and expired breath of rats during a 10-day post-exposure period. Examining radioactivity in the feces of rats treated with 2,3,7,8-TCDD, Rose et al. (1976) found label associated with compounds other than 2,3,7,8-TCDD. Although both of these studies indicate that metabolism had occurred, the metabolites were not isolated. Polar metabolites of 2,3,7,8-TCDD have been identified in the urine, feces and bile of rats and hamsters, but not in the adipose tissue or liver storage sites (Poland and Knutson, 1982). Poiger and Schlatter (1979) and Ramsey (1979) isolated and tentatively identified, as glucuronides of hydroxylated 2,3,7,8-TCDD, metabolites of 2,3,7,8-TCDD from the bile of rats dosed repeatedly with radiolabeled compound. Olson et al. (1980) collected urine and bile from hamsters treated with labeled 2,3,7,8-TCDD by gavage or intraperitoneal injection. On fractionation of the sample by HPLC, the radioactivity did not migrate with authentic 2,3,7,8-TCDD, indicating that metabolism occurred prior to excretion into these fluids. The enzymatic hydrolysis of the

2,3,7,8-TCDD metabolites from the rat and hamster altered the chromatographic profile of the metabolites, indicating the presence of glucuronide conjugates in bile and sulfate conjugates in urine (Olson and Bittner, 1983).

2,3,7,8-TCDD is a potent inducer of both liver and kidney microsomal enzymes (Beatty and Neal, 1976; Buu-Hoi et al., 1971, 1972; Fowler et al., 1975; Greig, 1972; Greig and DeMatteis, 1973; Hook et al., 1975a,b; Lucier et al., 1973, 1975a,b; Woods, 1973) and 2,3,7,8-TCDD metabolized by this system produces active products that can bind covalently to microsomal protein RNA and DNA (Guenthar et al., 1979; Poland and Glover, 1979). 2,3,7,8-TCDD metabolites collected from the bile of dogs, however, were 100 times less toxic than the parent compound when tested in guinea pigs, suggesting that the metabolism may also be part of a detoxification mechanism (Weber et al., 1982).

Recently, several metabolites of 2,3,7,8-TCDD have been identified. Sawahata et al. (1982) investigated the in vitro metabolism of 2,3,7,8-TCDD in isolated rat hepatocytes. The isolated metabolites were subsequently identified as 1-hydroxy-2,3,7,8-TCDD and 8-hyroxy-2,3,7-trichlorodibenzo-p-dioxin. Poiger et al. (1982) identified six metabolites in the bile of dogs that were given a lethal dose of [$^3$H]2,3,7,8-TCDD. The major metabolite was 1,3,7,8-tetrachloro-2-hydroxydibenzo-p-dioxin. 2,3,7,8-Trichloro-3-hydroxydibenzo-p-dioxin and 1,2-dichloro-4,5-hydroxybenzene were also identified as minor metabolites. The structures of the three remaining metabolites were not determined; however, two appeared to be trichlorohydroxydibenzo-p-dioxins and the third was apparently a chlorinated 2-hydroxydiphenyl ether. Olson and Bittner (1983) reported that the rate of 2,3,7,8-TCDD metabolite formation in vitro was higher in hepatocytes from the hamster than in hepatocytes from the rat. Qualitative evaluation of in vivo and in vitro metabolites by HPLC also suggested significant interspecies variability. The authors suggested that such differences in metabolism may partially explain the differences in toxicity among species.

## 4.4. EXCRETION

Fecal excretion (possibly entero-hepatic-derived) is the dominant route of elimination in rats (Piper et al., 1973; Rose et al., 1976; Van Miller et al., 1976) and monkeys (Van Miller et al., 1976), with urinary excretion eliminating only 0.25% of the amount removed in the feces. In rats receiving 1.0 µg/kg/day of 2,3,7,8-TCDD for 7 weeks, the males excreted only 3.1% of the dose in the urine, while the females excreted 12.5% of the total administered dose (Rose et al., 1976). Half-time of elimination of labeled 2,3,7,8-TCDD in rats was 31 ± 6 days and 23.7 days for single and repeated doses, respectively (Rose et al., 1976). Similar half-times of between 22 and 43 days were obtained in guinea pigs (Nolan et al., 1979). However, in hamsters, the half-life of elimination was between 10.8 and 5 days following treatment by gavage and intraperitoneal injection, respectively (Olson et al., 1980). The urine and feces accounted for the elimination of 35 and 50%, respectively, of the dose. In the rhesus macaque monkey, McNulty et al. (1982) reported that 2 years after administration of a single oral dose of 2,3,7,8-TCDD of 1 µg/kg, tissue levels of the compound were 100 ppt in adipose tissue and 15 ppt in the liver. These results indicate that prolonged retention of 2,3,7,8-TCDD may occur in this species.

Inter-strain differences have also been observed in the half-time of 2,3,7,8-TCDD in three mouse strains. These differences may be attributed to the fact that the DBA/2J strain possesses ~2-fold greater adipose tissue stores than the C57BL/6J and B6D2F$_1$/J strains (Gasiewicz et al., 1983).

The sequestering of the lipophilic toxin in adipose tissue of the DBA/2J mouse may contribute to the greater persistence of 2,3,7,8-TCDD in this strain.

Studies in the rat, guinea pig, hamster and mouse have found that all of the 2,3,7,8-TCDD-derived radioactivity excreted in the urine and bile corresponds to metabolites of 2,3,7,8-TCDD. The apparent absence of 2,3,7,8-TCDD metabolites in liver and fat suggests that once formed, the metabolites of 2,3,7,8-TCDD are readily excreted. Thus, urinary and biliary elimination of 2,3,7,8-TCDD is apparently dependent upon metabolism of the toxin. Although urine and bile appear to be free of unmetabolized 2,3,7,8-TCDD, data from the hamster and rat indicate that a significant amount (10-40%) of unchanged 2,3,7,8-TCDD may be excreted into the feces. Unmetabolized 2,3,7,8-TCDD thus appears to enter the intestinal lumen by some route other than bile for a number of days following treatment. These data suggest that the in vivo half-life for elimination of 2,3,7,8-TCDD may not directly reflect the rate of 2,3,7,8-TCDD metabolism in a given animal (Neal et al., 1982).

# 5. EFFECTS

## 5.1. CARCINOGENICITY

**5.1.1. Human Studies.** Observations of an unusual occurrence of relatively rare soft-tissue sarcomas were first made by Hardell (1977). Of some 87 patients seen from 1970-1976 at the Department of Oncology, University Hospital, Umea, Sweden, seven individuals with soft-tissue sarcomas were identified. All seven had had occupational exposure to phenoxy acids 10-20 years earlier. The tumors were 2 leiomyosarcomas, 1 liposarcoma, 1 rhabdomyosarcoma, 1 myxofibrosarcoma and 2 additional sarcomas of which the histopathology was uncertain but one was probably a neurofibrosarcoma and the other a rhabdomyosarcoma. The clustering of this rare tumor type among these patients prompted the author to suggest that epidemiological studies be done to determine if exposure to phenoxy acids and the impurities they contain are related to the occurrence of soft-tissue sarcomas.

Zack and Suskind (1980) reported a soft-tissue sarcoma death in a cohort study of workers exposed to 2,3,7,8-TCDD in a trichlorophenol process accident in Nitro, West Virginia. This tumor, a fibrous histiocytoma, was noted by the author as a rare event. This study, referred to as the Nitro study, is discussed later.

Cook et al. (1980) in a cohort mortality study of 61 male employees of a trichlorophenol manufacturing area, who exhibited chloracne following a 1964 exposure incident, noted four deaths by the end of his study period, one of which was due to a fibrosarcoma. The authors did not seem to attribute any special significance to this finding at the time.

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*Portions of this section were taken from U.S. EPA (1980c).

Ott et al. (1980) in a cohort mortality study of 204 employees exposed to 2,4,5-T during its manufacture from 1950 to 1971, found no soft-tissue sarcomas among 11 deaths that had occurred by 1976. One of these 11 deaths was due to a malignant neoplasm.

In a review of the studies of Zack and Suskind, Cook, an unpublished study by Zack (in which a liposarcoma was found), and a study by Ott et al. (1980) and Honchar and Halperin (1981) noted 3 (2.9%) soft-tissue sarcomas in a total of 105 deaths. Among United States males aged 20-84, 0.07% of the deaths were reported as soft tissue sarcomas (ICD 171, 8th Revision, 1975)* indicating an unusual excess of such tumors. This may be somewhat of an underestimate because of the possiblity that some soft-tissue sarcomas may be coded to categories other than ICD 171. Individually, none of the reported case studies reported a significant excess of soft-tissue sarcomas. Cook (1981a) found an additional malignant fibrous histiocytoma after a later review of the medical records from his earlier cohort study. Cook, who was familiar with the three earlier cases, noted that frank chloracne occurred previously in two cases of the four having a diagnosis of malignant fibrous histiocytoma. A third person diagnosed as having a fibrosarcoma worked in a trichlorophenol (TCP) process area contaminated with 2,3,7,8-TCDD. This individual exhibited facial dermatitis but no diagnosis of chloracne. The fourth case (diagnosed as a liposarcoma) had been employed earlier in a plant producing 2,4,5-T. Cook noted that although chloracne was not reported, it could not be discounted. He also noted that all four were cigarette smokers and suggested that smokers with chloracne caused by 2,3,7,8-TCDD exposure may be subject to an increased risk of fibrous soft-tissue sarcomas although no prior reports have shown soft tissue sarcomas associated with cigarette smoking.

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*Department of Health, Education, and Welfare. U.S. Public Health Service. National Center for Health Statistics of the United States, 1974. Vol. II. Mortality, Part A.

Hardell and Eriksson (1981) discounted this hypothesis by citing that only one of Hardell's seven cases exhibited chloracne before the appearance of the soft-tissue saromcas, and that in their subsequent later case control study, they found no difference in smoking habits between his cases and controls.

Moses and Selikoff (1981) reported a fifth soft-tissue sarcoma in a worker employed at the Monsanto Chemical Company at a time when trichlorophenol and 2,4,5-T were being produced. He died of a retroperitoneal neurogenic sarcoma (malignant schwanoma) in 1980 at the age of 58. The employee, before his death, in a detailed occupational history said that he believed he was exposed to these chemicals while he was a truck driver, hauler and maintenance worker, but that he did not work in the production of either chemical. He was a nonsmoker and did not have a history of chloracne.

Johnson et al. (1981) treated a father and son with soft-tissue sarcomas (the 33-year-old son was diagnosed as having a fibrosarcomatous mesothelioma, while the 53-year-old father had a liposarcoma). Both were exposed to halogenated phenol derivatives. The author noted that 2,4-dichlorophenol can be a precursor of 2,4-D and 2,4,5-T. The father had had prolonged exposure before his disease. The son supposedly had a shorter latency, according to the author. In neither case is the follow-up time given. The exposure of the son is not likely related to that of the father.

Sarma and Jacops (1981) reported three cases of thoracic soft-tissue sarcoma in individuals who were presumably exposed to Agent Orange while serving in Vietnam. The diagnoses were fibrous histiocytoma, mediastinal fibrosarcoma, and a pleural/diaphragmatic leiomyosarcoma. All three served in areas where defoliants were used at the time. One was drenched with the material in one spraying.

Bishop and Jones (1981) found two cases of non-Hodgkin's lymphomas of the scalp in a related clinical study of 158 employees of a pentachlorophenol manufacturing plant in Wales. Homologues of 2,3,7,8-TCDD occurred as contaminants at up to 300 ppm at intermediate manufacturing stages and 5 ppm in the final products. Mild, moderate and severe cases of chloracne were seen in many employees, including the two men who subsequently developed lymphomas. Both men worked in processes where exposure to other chemicals occurred, including exposure to aromatic hydrocarbons. The authors reported that only 0.28 tumors of this type could be expected to occur in a group of 158 workers (ICD 200 and 202), although the basis for the computation of expected numbers is not stated.

Olsson and Brandt (1981) noted that of 123 male patients seen at their clinic in Sweden with a recent diagnosis of non-Hodgkin's lymphoma (NHL), 5 had cutaneous lesions as the only clinically detectable manifestation of NHL. Four of the five were reported to have repeatedly sprayed large areas with phenoxy acid herbicides. In the remaining 118 NHL patients, only seven had a similar occupational exposure to phenoxy acids. The authors reported this to be significant at $p<0.001$. Olsson and Brandt suggested that a relationship exists between cutaneous presentation of NHL and occupational exposure to phenoxy acids, and believe their observations were similar to those of Bishop and Jones (1981).

The total number of workers with these illnesses who were exposed to phenoxy acids and/or chlorophenols is small, but considering the rarity of this cancer, it is unusual that so many cases of soft-tissue sarcomas have occurred. A Lancet editorial (Anonymous, 1982) calls this phenomenon "disturbing."

**5.1.2. Soft-Tissue Sarcomas.** Soft-tissue sarcomas (STS) constitute a collection of heterologous lesions that include both malignant and nonmalignant tumors. Not all of them have their origin in primordial mesenchymal cells. Some exceptions are tumors of peripheral nerves, and neuroectodermal tumors which are classified as STS, but are derived from nonmesenchymal cells. Classification, grading and staging of STSs is difficult because of the capacity of such cells to differentiate into many different tissues. Fairly precise histogenetic classification of such tumors is accomplished through consideration of growth patterns and cell morphology and evaluation of intracellular and extracellular products of tumor cells. There are a dozen distinctly different classes of mesenchymal cells that develop into the following six well-defined tissue complexes: fibrous tissue, tendosynovial tissue, adipose tissue, muscle, vessels and bone. STSs can be induced in any of these tissue types (Hajdu, 1983). The classification of STSs for cause of death coding in the ninth and latest revision of the International Classification of Diseases (ICD, 1975) places STSs into one of several categories. But chiefly, they fall into "malignant neoplasms of connective and other soft-tissue" (ICD 171). Lymphosarcomas, retroperitoneal sarcomas and extra skeletal STSs of the bone are coded elsewhere. In some instances, if site is mentioned, it is coded to the site, i.e., leiomyosarcoma of the stomach (ICD 151.9), neurofibroma of the chest wall (215.4).

Questions have been raised concerning the appropriateness of lumping together malignant tumors of different sites and tumor types in order to derive risk estimates. It may not be scientifically appropriate to do so because an elevated risk cannot readily be ascribed to a particular site or type as is usual with most carcinogenic chemicals and substances. Unfortunately, with respect to STSs, tallies of deaths from STSs of particular sites and types are not maintained separately by the vital statistics offices because of their rarity, and therefore, it is impossible to derive risk estimates for particular types at given sites. Altogether, ~2000 deaths/year can be attributed to STSs in the United States, most of which are coded to ICD category 171 for purposes of developing incidence and mortality rates for this composite cause. Within ICD 171, individual types that may be correlated with exposure cannot be identified.

A separate problem that potentially could arise from assigning STSs to multiple ICD codes is that incidence and death rates from STSs may be underestimated. Furthermore, risk estimates derived from dividing observed cases (or deaths) by expected cases (or deaths) could be biased upward. This could happen when observed STSs classified to ICD codes other than ICD 171 are lumped together while expected STSs are based upon ICD 171 only. Thus, action of this sort, especially with respect to cohort studies of individuals exposed to dioxin-containing herbicides and/or chlorophenols, could lead to risk estimates that may be biased upward by the inclusion of STSs in the observed category for risk estimation that should be coded to categories other than 171.

Prompted by clinical observations over a 7-year period of malignant sarcomas in seven men with previous occupational exposure to phenoxyacetic acid herbicides (Hardell, 1977), researchers at the Department of Oncology, University Hospital, Umea, Sweden, initiated case-control epidemiologic studies (Cole, 1979) to test the hypothesis of an etiologic association (Hardell and Sandstrom, 1979). Cases were defined as male patients with sarcomas of soft connective tissue, such as smooth muscle (leiomyosarcoma) and fat (liposarcoma). The distribution of tumor types in the two studies is shown in Table 5-1. Sarcomas of tissues, such as bone and cartilage, were excluded as cases. According to the authors, these tumors may have a different etiology and there occurred a different age-distribution in patients with these tumors as compared with that of STS (Hardell, 1983).

Two case-control studies were conducted: the first in northern Sweden (referred to below as Study A) and the second in the southern part of the country (Study B). The exposures to the substances of primary interest are shown in Table 5-2. In the north (Study A), occupational exposure to phenoxyacetic acids took place in both forestry and agricultural work. In the south (Study B), these exposures were predominantly agricultural. The phenoxyacetic acids to which exposure occurred consisted predominantly of 2,4,5-T and 2,4-D in both studies. Exposure to 2,4,5-T in the absence of 2,4-D was rarely reported in either study. Exposure to chlorophenols, which contain chlorinated dibenzodioxin impurities (Levin et al., 1976) occurred mostly in sawmill work and paper pulp production. Very few persons reported exposure both to phenoxyacetic acid and chlorophenols in these studies. Of the two predominant phenoxyacetic acids, only 2,4,5-T is known to be contaminated with 2,3,7,8-TCDD. In Study B, a relative risk of 4.9 (90% confidence intervals 1.6-11.1) was found in relation to exposure to phenoxyacetic acid herbicide other than 2,4,5-T (2,4-D, MCPA, mecoprop, dichloroprop).

## TABLE 5-1

## Distribution of Tumor Types in Two Case-Controls Studies of Soft-Tissue Sarcoma

| Diagnosis | Tissue of Origin | Percent of Cases | |
|---|---|---|---|
| | | Study A[a] (n=52) | Study B[b] (n=110) |
| Leiomyosarcoma | Smooth muscle | 30 | 23 |
| Fibrous histiocytoma | Subcutaneous connective tissue | 17 | 25 |
| Liposarcoma | Fat tissue | 14 | 6 |
| Neurogenic sarcoma | Nerve tissue | 10 | 4 |
| Angiosarcoma | Blood vessels | 8 | 2 |
| Myxosarcoma | Primitive connective tissue | 6 | 8 |
| Fibrosarcoma | Fibrous tissue | 4 | 8 |
| Other sarcomas | | 11 | 24 |
| Total | | 100 | 100 |

[a] Unpublished information supplied by Hardell to EPA (Hardell and Sand strom, 1979)

[b] Eriksson et al., 1979, 1981

## TABLE 5-2

### Exposure Frequencies in Two Case-Control Studies of Soft-Tissue Sarcoma

| Substance(s) | Percent Exposed | | | |
|---|---|---|---|---|
| | Study A | | Study B | |
| | Cases (n=52) | Controls (n=206) | Cases (n=110) | Controls (n=219) |
| Phenoxyacetic acids only | 23.1 | 6.3 | 12.7 | 2.3 |
| Chlorophenols only | 11.5 | 2.4 | 10.0 | 3.6 |
| Both | 1.9 | 0.5 | 0 | 0 |
| Total | 36.5 | 9.2 | 22.7 | 5.9 |

*Sources: Study A, Hardell and Sandstrom, 1979; Study B, Eriksson et al., 1979, 1981

Relative risks in relation to the three major categories of exposure are shown in Table 5-3.* Studies A and B indicate a risk of developing STSs among workers exposed to phenoxyacetic acids only, chlorophenols only, or phenoxyacetic acids and/or chlorophenols several times higher than among persons not exposed to these chemicals. In each comparison, a high relative risk is estimated and was thus unlikely to have resulted by chance alone.

Since little is known of the etiology of STSs, the consideration of confounding in these studies was largely a hypothetical matter. The authors prevented the effects of age, sex, and place of residence as possible confounding factors in the selection of controls.† Because of the high correlation between exposure to the substances of interest and employment in agriculture and forestry, a possible alternative hypothesis could be that some other unknown factor present in these occupations was responsible for the elevated relative risks.

To test this hypothesis, it is possible to calculate the relative risk in relation to the phenoxyacetic acid exposure in Study B, restricting the analysis to workers within agriculture and forestry. The result is a relative risk of 6.1 (90% confidence interval 2.4-15.4). This finding suggests that a confounding risk factor for STS distributed throughout agriculture and forestry work was not responsible for the overall increase in risk found in relation to phenoxyacetic acid exposure.

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*In the analyses considering phenoxyacetic acids only and chlorophenols only, persons exposed to the other categories of substances were excluded. In Study A, the three persons exposed to both chlorophenols and phenoxy acetic acids were included in all comparisons.

†Controls were matched individually to cases on the basis of these factors. Unmatched analyses are presented in Table 5-3 for the sake of simplicity. The matched-method relative risks for exposure to phenoxyacetic acids and/or chlorophenols were 6.2 ($p<0.001$) in Study A and 5.1 ($p<0.001$) in Study B.

## TABLE 5-3

Relative Risks of Soft-Tissue Sarcoma in Relation to Exposure to Phenoxyacetic Acids and Chlorophenols in Two Case-Control Studies[a]

| | Phenoxyacetic Acids Only | | Chlorophenols Only | | Phenoxyacetic Acids and/or Chlorophenols | |
|---|---|---|---|---|---|---|
| | Study A | Study B | Study A | Study B | Study A | Study B |
| Relative risk[b] | 5.3 | 6.8 | 6.6 | 3.3 | 5.7 | 4.7 |
| 90% Confidence interval[c] | 2.7-10.2 | 3.1-14.9 | 2.8-15.6 | 1.6-7.0 | 3.2-10.2 | 2.7-8.3 |
| Significance level[d] | <0.001 | <0.001 | <0.001 | <0.005 | <0.001 | <0.001 |

[a] Source: Study A, Hardell and Sandstrom, 1979; Study B, Eriksson et al., 1979, 1981

[b] Unmatched odds ratio

[c] Test-based method of Miettinen, 1976

[d] Chi square statistic, no continuity correction, one-tailed test

Because exposure histories were obtained by means of questionnaires and interviews, the major potential source of bias in these studies stems from the need to rely upon the personal recollection of cases and controls for exposure histories. The published papers indicate that the researchers paid a great deal of attention to this potential problem and specific efforts were made to avoid it during the conduct of the study.

In addition, the relative risk calculated by considering the agriculture and forestry workers who did not report exposure to phenoxyacetic acids or chlorophenols and comparing them to unexposed persons in other occupations was 0.9 (90% confidence interval 0.3-2.4) in Study B. This suggests that little recall bias was present (Axelson, 1980).

In an update of their earlier study, Eriksson et al. (1981) obtained information on the effects of phenoxy acids in the absence of the impurities--polychlorinated dibenzodioxins and dibenzofurans. The risk ratio given exposure to phenoxy acids free of polychlorinated dibenzodioxins and dibenzofurans equaled 4.2 based upon 7 out of 14 respondents who indicated exposure to phenoxy acid herbicides. When consideration was given to persons exposed only to phenoxy acids that contain such impurities, the relative risk was 17.0. A description of the basis for the determination of exposure or non-exposure to dioxins is not well presented in this study.

The author concluded that exposure to phenoxy acids and chlorophenols "might constitute a risk factor in the development of soft-tissue sarcomas." This risk relates not only to 2,4,5-trichlorophenoxy acids containing dioxin impurities, but to other phenoxy acids as well. Some doubt was raised concerning the possible misclassification of individuals who were exposed to phenoxy acids free of polychlorinated dibenzodioxins (i.e., in particular, "dichoroprop" in the Eriksson study). In a recent communication from

Hardell (1983), Eriksson recalculated his risk estimates after reclassifying his dichoroprop-exposed cases and controls into the category of probable exposure to phenoxy acids contaminated with polychlorinated dibenzodioxins and removing them from the nonexposed category. His new estimates were 4.0 based upon 5 out of 8 respondents who were exposed to phenoxy acids free of contamination and 10.9 for those exposed to contaminated phenoxy acid. The first estimate was of only borderline significance utilizing the Mietinen test based statistic, thus, weakening any finding that the risk of STS extends to phenoxy acids free of dioxin.

In a cohort mortality investigation Cook et al. (1980a) studied 61 males involved in a 1964 exposure incident who had absorbed 2,3,7,8-TCDD through the skin and developed chloracne. The skin lesions characterizing chloracne ranged from a few comedones on the back of one employee (predating his entry into the process area where exposure could occur) to severe cysts and comedones over the faces, scalps, ears, necks and backs of the remaining employees of the group. Since the main route of exposure was not through the respiratory tract, no measurements of dioxin in the air were provided by the author. On the other hand, the author did divide the cohort of 61 males into potentially "high" vs. "low" exposure by place of work based upon dermal exposure, although not stated. Vital status was traced from the data of the incident through 1978. Altogether only 4 deaths were observed by the end of the follow-up, vs. 7.8 expected. Of these, 3 were cancer deaths vs. 1.6 expected. The remaining death was hypersensitive heart disease vs. 3.8 expected. The histopathologic causes of death of the three cancer victims were 1) fibrosarcoma, 2) glioma with metastases, and 3) adenocarcinoma. The authors report that all three victims smoked a minimum of one pack of cigarettes a day for "many years." Not enough information is provided by the authors to conclude that any of these four deaths were smoking related. Site of tumor is not mentioned in the cancer deaths.

Cancer mortality is slightly elevated in this cohort. The study has low sensitivity and lacks a sufficient latent period. This increased mortality was not attributable to any particular cause and no deaths were attributable to liver cancer. Additionally, the authors state that only one of the cancer deaths possessed "documented" evidence of chloracne, although this appears to be at variance with the definition of the cohort, which was reported by the authors to consist of males who reported to the medical department with skin conditions subsequently "diagnosed as chloracne." The authors concluded that the latency period was sufficient to "allow the identification of a potent human carcinogen," since it "exceeded 14 years." Orris (1981) noted that in the Hardell and Sandstrom (1979) study the authors stated that the latent period for soft-tissue tumors may be as long as 27 years and for many, over 14 years. In any case, Hueper and Conway (1964) noted that the latent period for the chemical induction of solid malignant tumors in man exceeds 15 years and is probably <30 years.

Smith et al. (1982b) conducted an initial case-control study of 102 males identified from the New Zealand Cancer Registry as having STSs (ICD 171) between 1976 and 1980. For each case, three controls each with another form of cancer were matched by age and year of registration. The selection of cancer controls from the same registry was done to eliminate recall bias and/or interviewer bias. The distribution of histological types in the cases is given in Table 5-4. An interview to elicit occupational history information was accomplished via the telephone either with the next of kin to the patient or the patient himself if he was well enough, although the information was not used in this preliminary analysis.

## TABLE 5-4

### Distribution of Histological Types of Soft-Tissue Sarcomas*

| Cell Type | Number of Cases | Percent |
|---|---|---|
| Fibrosarcoma | 25 | 24 |
| Liposarcoma | 20 | 20 |
| Rhabdomyosarcoma | 9 | 9 |
| Leiomyosarcoma | 7 | 7 |
| Malignant Histiocytoma | 6 | 6 |
| Other | 22 | 21 |
| Unspecified | 13 | 13 |
| Total | 102 | 100 |

*Source: Smith et al., 1982b

Comparisons between cases and controls were accomplished by use of occupational groupings according to the Standard Classification System of New Zealand focusing on those occupational groups with a potential for exposure to phenoxy herbicides and chlorophenols. Expected cases for each major occupational classification were derived based upon the occupational distribution of the controls. The authors found no unusual excess of cases of STS in any major occupational category. In agriculture, forestry and fishing, 14 cases were observed vs. 14.0 expected. In laborers, production and transport workers, 35 cases were observed vs. 37.0 expected. A further breakdown of these two broad categories into finer subcategories within the major occupational categories revealed no significant excesses. The study, however, is not useful in assessing the risk of STS from exposure to phenoxy acids and/or chlorophenols for several reasons. First, as was pointed out by the authors but subsequently dismissed by them as having not much of an influence, is the possibility that movement from one major occupational category to another over the time period involved for latent conditions to manifest themselves could introduce a negative bias into any estimates of relative risks. The latency for STS was suggested to be a minimum of 15 years (Hueper and Conway, 1964).

The finding of no switching from one occupational category to another that was noted in the "first 20 interviews" in which a change could be noted is not necessarily indicative of fidelity to the same job over long periods in all 408 cases and controls. Information identifying a change may be lacking in those cases and controls if in fact one did occur possibly because of several reasons, e.g., separation of the earlier work history from the latter; purging of earlier employment records, etc. Besides the "first 20 interviews" where a change could be noted is not necessarily representative of the entire cohort in any case.

Furthermore, the authors do not know absolutely that any of their cases and controls were exposed to phenoxy acids and/or chlorophenols since apparently no effort was made to confirm "potential" exposures. Only differences in occupational classification were noted where "potentially" cases or controls could have had exposure to the dioxin-containing herbicides. It was pointed out that the risk estimates noted do not "preclude" the possibility that an association may be found in this study when the cases and controls (or surviving kin) are interviewed for chemical spraying at a later time. The authors themselves conclude that the preliminary study results "should not be taken as substantial evidence against the hypothesis that phenoxy herbicides and chlorophenols may cause human cancer."

The distribution of tumor types differed considerably from the Hardell and Eriksson study to the Smith study. Leiomyosarcomas, malignant histocytomas, neurogenic sarcomas and myxosarcoma seem to predominate in the Hardell and Eriksson study, whereas fibrosarcomas and liposarcomas appear prominently in the Smith study. More attention should be devoted to the study of the distributions of STS types in registry data everywhere in order to determine if such variations in the reporting of STS types are random occurrences. It is possible that the cancer effect of exposure to phenoxy herbicides may be narrowed to just certain types of STSs, the predominant ones in the Swedish studies.

In a later study of STSs, Smith et al (1983a) conducted a case-control study of STSs in males that were reported to the New Zealand Cancer Registry by Public Hospitals between 1976 and 1980. The author matched one cancer control randomly chosen from the registry with each case, initially starting with 112 of each. Controls were matched for year of registration and by date of birth ± 2 years. Inquiries were made by the authors with the hospital consultant, family doctor, and finally the next-of-kin or patient if alive. Telephone interviews were conducted by only one interviewer who had no knowledge of the patient's cancer history and were completed on 80 cases and 92 controls. Because some 32 potential cases (14 ineligible) and 20 controls were excluded or lost from the study for various reasons, it raises a question whether control of confounding by age and year of registration was maintained in the final group of 172 cases and control included in the analysis. Presumably the corresponding "matched" case or control to each of the 52 lost members of the total study group were not excluded. However, since the span of registration was only 5 years, not much age confounding could occur.

Patients were classified as having had potential exposure to phenoxyacetic acids if they had definite, probable or possible exposure to phenoxyacetic acid through spraying or hand contact. The actual chemical was identified only in some instances. The authors concluded in all remaining situations that if the member sprayed "gorse" and/or "blackberries" this was tantamount to potential exposure to phenoxyacetic acid. Smith calculated elevated but nonsignificant relative risks of exposure to phenoxyacetic acid ranging from 1.3 in those individuals who were "probably exposed" for a minimum of 5 days not in the previous 10 years before cancer registration to 1.6 in individuals "probably exposed" for a minimum of 1 day not in the previous 5 years before cancer registration. When risk ratios were calculated after stratifying by year of birth and whether or not the patient or a relative was interviewed, the rates increased to 1.7 (from 1.6) in the latter and 1.4 (from 1.3) in the former calculation, although still nonsignificant. If the numbers would allow, it would be of interest to repeat the above calculations excluding only those with potential exposure occurring only within the 15-year period just before cancer registration. The small numbers that remain following the 15-year lapse probably precludes such an analysis. Furthermore, the categories of exposure "probably or definitely" exposed for $\geq$1 day or even 5 days raises a question whether any of the cases or controls could really be said to have ever come in contact with enough phenoxyacetic acid to justify such a designation. It could be that, in fact, potentially exposed individuals in New Zealand have had little or no contact with the herbicide.

The authors did conclude that the finding of a relative risk of 1.7 in individuals with $\geq$1 day exposure not in the last 5 years cannot be entirely discounted. But then the authors state that if exposures of $\geq$5 days prior to 10 years before cancer registration are not included they would expect an increase, and since they do not see an increase, there is no evidence of a "real causal link." One might ask whether this is a suitable criterion for providing evidence of a causal association. Perhaps a more valid group for study would be one where the potential exposure was considerably longer than "5 days" and >15 years before initial cancer registration. As kind of a subtle justification for the finding of no significant risk in workers exposed in phenoxy acids, the author alludes to the fact that there are currently 500 full-time workers registered in New Zealand who do full time ground spraying and altogether some 2000 workers who were at some time professionally involved in phenoxyacetic acid herbicide spraying from the air or ground with exposure "very much greater" than that of patients in this study. This kind of argument has appeal if these workers could be shown to have had their exposure sufficiently far in the past that latency considerations could be adequately addressed. However, the real question again remains how much real exposure did those patients in the study really have 10-15 years earlier, and in what numbers. The author remarks that it is surprising that he found no STS victims who had ever worked full-time in phenoxyacetic acid herbicide spraying. Perhaps they have not yet been observed for a long enough period. However, as was pointed out by the author, the findings do not support the hypothesis that exposure to phenoxyacetic acid herbicides causes STS. But neither do they support a negative finding without better documentation regarding actual exposure and time of actual exposure. The author does note (Smith, 1983), however, that his documentation of exposure to 2,4,5-T (and 2,4-D) is at least as good as that in the Hardell study, and that although Hardell noted higher relative risks of <30 days exposure, Smith did not. Hence the paradox. Smith does admit the possibility that 2,3,7,8-TCDD contaminations might be lower in New Zealand as opposed to 2,3,7,8-TCDD contamination in the Swedish studies, although there is no evidence for it. He still maintains that his study shows that exposure to phenoxyacetic acids may not be associated with STS.

Pazderova-Vejlupkova et al. (1981) studied 80 workers involved in the production of 2,4,5-sodium trichlorophenoxyacetate and butylester of trichlorophenoxyacetic acid who subsequently became ill from exposure to 2,3,7,8-TCDD during the period 1965-1968. Only 55 members of this group were followed for 10 years. The remaining 25 either refused participation or moved leaving no forwarding address. Most patients developed chloracne while 11 developed porphyria cutanea tarda. Chief chemical signs were metabolic disturbances, pathologically elevated lipids with abnormalities in the lipoprotein spectrum, and "pathological" changes in glucose tolerance. Other symptoms noted were biochemical deviations consistent with "a mild liver lesion," light steatosis, periportal fibrosis or activation of Kupffer cells, or nervous system focal damage (peripheral neuron lesion in lower extremities). Altogether six patients were reported to be deceased during this 10-year period, 2 from bronchogenic carcinoma, 1 from cirrhosis, 1 atherosclerosis precipue cerebi and 2 in auto accidents. No STSs or lymphomas were found. Since there was no comparison population with which to estimate relative risk for cancer, the study must be classified at best as clinical with respect to cancer. The six deaths that occurred during the 10-year observation period in the 55 cannot be construed to be associated with exposure to the 2,4,5-T. Because of the small number of cases and the short follow-up period, nothing can be said concerning the association of exposure with cancer, especially specific types of cancer such as STS or non-Hodgkin's lymphoma.

Riihimaki et al. (1982, 1983) studied a cohort of 1926 herbicide applicators formed in 1972 from personnel records of four Finnish employers (e.g., the Forestry Authority, Highway Authority, State Railways and a state-owned electric power company). Chlorinated phenoxyacids had been used since the 1950's in Finland for spraying. They constituted 2:1 mixtures of emulsified esters of 2,4-D and 2,4,5-T dissolved in water. Analyses from old herbicide formulations dating back to the 1960's revealed that these mixtures contained 0.1-0.9 mg/kg of 2,3,7,8-TCDD).

This cohort of male workers was exposed a minimum of 2 weeks during at least one growing season from 1955-1971. Follow-up continued 9 years through 1980 for mortality but only until 1978 for morbidity. Fifteen individuals could not be traced by 1980. Expected deaths were generated based upon cause- and age-specific national Finnish death rates for 1975. Expected cases were similarly calculated based upon national incidence rates of 1975.

By 1980, 144 deaths had occurred vs. 184.0 expected, a deficit of 22% in observed mortality. Only 26 cancer deaths had occurred vs. 36.5 expected, a 29% deficit. The authors separated out "natural" deaths from the total. The observed residual deaths equaled 39 while the expected deaths equaled 28.7. This excess was of borderline significance. The authors also considered 10-year and 15-year latent periods. Even after 15 years, the deficit of deaths continued to manifest itself both in categories of all causes and total cancers; 35 observed vs. 53.6 expected and 5 observed vs. 11.3 expected, respectively. Similarly, the 7-year follow-up of cancer morbidity revealed 26 cases of cancer vs. 37.2 expected. After a 10-year latent period, 16 cancer cases were observed vs. 20.1 expected. None of the 26 cancer deaths or 26 cancer cases were of the STS or lymphoma type. (However, only 0.1 STS and 0.5 lymphomas were expected.) In no instance was cancer of any site significantly elevated.

The authors note that this unusual deficit of mortality and morbidity of between 70 and 82% (even after 15 years from initial exposure) is probably a consequence of the "healthy worker effect" in that only able-bodied and healthy individuals were selected into the industry. The fact that the cohort was assembled in 1972 from records of persons who were exposed as early as 1955 (17 years prior) raises the likelihood that in 1972 a "survivor" population remained (45 deaths before 1972 were eliminated from the cohort) that was relatively healthy. Furthermore, the unusually large number of not "natural" expected and observed deaths (probably accidents and external causes) occurring to this cohort indicate a relatively youthful population was under scrutiny. The leading cause of death to persons under 35 years is from accidents, based on national vital statistics.

The authors correctly note that, because of limitations in the study material, only powerful carcinogenic effects could be detected. Risk ratios higher than 1.5 for all cancers, 4.0 for lymphomas and 10.0 for STS could be excluded based on this data set from the authors own calculations. More follow-up is needed in order to provide a stable assessment of the relationship between exposure and cancer. The authors concluded that this study will allow no assessment of STS because "the number of persons having a sufficiently long latency period is too small." It was suggested that more valid conclusions could be made only with the passage of time.

Recently, the Michigan Department of Public Health (1983b), produced an ecological study of soft and connective tissue cancer mortality rates in Midland and other selected Michigan counties. They found that mortality rates for this cause were 3.8-4.0 times the national average for the periods 1960-1969 and 1970-1978, respectively, for white females in Midland. These estimates are based upon 5 deaths and 7 deaths, respectively, and are listed in Table 5-5. No excess risk was reported among white males, however. The Michigan Department of Health concluded that because of the occurrence of these two successive elevated rates, it is unlikely to be a chance happening. At the same time the age-adjusted male and female cancer mortality rates for Midland were below that of the State of Michigan in the period 1970-1979. Midland County is the home of a major chemical company that produced phenoxyacetic acid herbicides until recently. The authors state that a detailed review of death certificates, hospital records, residency and occupational histories of the 20 male and female cases revealed no "commonalities" suggesting a "single causative agent" although a majority or their spouses had worked at this chemical facility. They recommend that a casecontrol study should be instituted to evaluate possible influences, such as lifestyle, occupation or location of residence on the risk of STS.

## TABLE 5-5

### Midland County Soft and Connective Tissue Cancer Deaths 1960-1981*

| Identification | | | Type of Malignancy | | | |
|---|---|---|---|---|---|---|
| Year of Death | Sex | Age | Type | Primary Site | Metastases | Month and Year Diagnosed |
| 1961 | F | 24 | Hemangiosarcoma | Face | Skull and upper lobe of lung | 5-58 |
| 1963 | F | 75 | Liposarcoma | Right gluteal | Unknown | Unknown |
| 1964 | F | 51 | Leiomyosarcoma | Uterus | Widespread | 11-63 |
| 1968 | F | 37 | Liposarcoma | Spine | Lungs, pelvis | 1-66 |
| 1969 | F | 45 | Fibrosarcoma | Right thigh | Lung, liver | 10-68 |
| | | | Leiomyosarcoma | Uterus | Adrenal gland and skin | |
| 1970 | F | 59 | Kaposi sarcoma | Right leg | Lymph nodes | 8-68 |
| 1970 | F | 56 | Fibrosarcoma | Right thigh | Spine | 1960 |
| | | | Leiomyosarcoma | Abdominal wall | Lung | 1967 |
| 1974 | F | 1 | Rhabdomyosarcoma | Inguinal area | Unknown | 8-73 |
| 1976 | F | 77 | Liposarcoma | Right thigh | Buttock, lung, rib, lymph nodes | 12-74 |
| 1978 | F | 64 | Leiomyosarcoma | Left knee | Liver, lymph nodes, lung, bone | 7-70 |

## TABLE 5-5 (cont.)

| Identification | | | Type of Malignancy | | | |
|---|---|---|---|---|---|---|
| Year of Death | Sex | Age | Type | Primary Site | Metastases | Month and Year Diagnosed |
| 1978 | F | 26 | Rhabdomyosarcoma | Rectum | Lung, neck, inguinal region | 6-76 |
| 1978 | F | 88 | Fibrosarcoma | Right cheek | Facial area | 6-78 |
| 1979 | F | 27 | Leiomyosarcoma | Left thigh | Lung | 3-78 |
| 1962 | M | 63 | Rhabdomyosarcoma | Left lower leg | Lung and right outer chest wall | 8-61 |
| 1967 | M | 77 | Mesothelioma | Lung | Lung, peritoneum and diaphragm | 6-67 |
| 1967 | M | 20 | Rhabdomyosarcoma | Pharynx | Periorbital area and liver | 1-67 |
| 1969 | M | 32 | Liposarcoma | Left arm | Perineum and buttock | 6-64 |
| 1971 | M | 76 | Leiomyosarcoma | Small intestine | Liver | 10-69 |
| 1972 | M | 89 | Leiomyosarcoma | Retro-peritonal region | Hepatic system | 7-72 |
| 1976 | M | 53 | Fibrosarcoma | Peritioneum | Lung, liver | 3-75 |

*Source: Michigan Department of Public Health, 1983b

In a separate review of the epidemiological evidence for STS from exposure to 2,4,5-T-containing herbicides, the United Kingdom Ministry of Agriculture, Fisheries and Food (1983) concluded that there was no evidence to recommend altering their earlier conclusion that formulations of phenoxy acid herbicides and related wood preservatives as "presently cleared" are safe and may continue to be used. This report readily discounts the positive studies of Hardell and Eriksson as being biased, and it makes no reference to the later validity study by Hardell (1981) of his own work utilizing colon cancer controls (see Section on Malignant Lymphoma). In this report Hardell answered these early criticisms that were reiterated by the British in their report. At the same time, the British report appears to put undue emphasis on nonpositive studies that do not demonstrate a risk, although most of them have methodological limitations (e.g., low power, insufficient latency and inappropriate study method). In short, the British review appears to be overly optimistic about the safety of 2,4,5-T herbicides.

In summary, the associations reported in the two Swedish soft-tissue sarcoma studies are strong enough to make it unlikely that they have resulted entirely from random variation bias or confounding, even though the possibility cannot be excluded. These studies provide a strong suggestion that phenoxyacetic acid herbicides, chlorophenols or their impurities are carcinogenic in humans.

5.1.3. **Malignant Lymphoma**. A separate series of clinical observations at the Department of Oncology in Umea, Sweden (Hardell, 1979), led the researchers to conduct a case-control study of malignant lymphoma in relation to phenoxyacetic acid, chlorophenols, and other organic compounds (Hardell et al., 1980, 1981). Approximately 33% of the cases in this study were patients with Hodgkin's disease; the remainder of the cases were non-Hodgkin's lymphomas.

This study employed essentially the same methods and produced results comparable to those of the STS studies: statistically significant 5-fold to 6-fold relative risks in relation to phenoxyacetic acids and chlorophenols. In addition, an elevated relative risk was found in connection with exposure to organic solvents, such as benzene, trichloroethylene, and styrene. In the published report, the methods and results were incompletely documented, especially the possibility of confounding by exposure to the organic solvents.

In the update of the earlier 1980 study, Hardell et al. (1981), utilizing the same basic data source, found that 36.1% of the cases had been exposed to phenoxy herbicides or chlorophenols, while only 9.6% of their controls were so exposed. The estimated relative risk was 6.0 when matching was considered and 5.3 when matching was eliminated. When cases and controls who were exposed to chlorophenols only were excluded, the relative risk of lymphoma from phenoxy acids alone was 4.8 (95% C.I. 2.9-8.1). On the other hand, if exposures to phenoxy acids are excluded and consideration is given to just chlorophenols (which includes combined exposure to phenoxy acids and chlorophenols), then the relative risk equaled 4.3 (95% C.I. 2.7-6.9). The author further subdivided this group into "low-grade" vs. "high-grade" exposures to chlorophenols. A continuous exposure of not more than 1 week or repeated intermittent exposures totaling not more than 1 month was classified as low-grade. The relative risk for high-grade exposure was 8.4 (95% C.I. 4.2-16.9), while that for low-grade exposure equaled 9.2 (95% C.I. 1.6-5.2). If exposure to organic solvents is examined, given that cases and controls exposed to only phenoxy acids and/or chlorophenols were excluded except for combined exposure to organic solvents, it is found that high-grade and low-grade relative risks were 2.8 (95% C.I. 1.6-4.8) and 1.2 (95% C.I. 0.5-2.6), respectively. However, the author notes that exposure to phenoxy acids and high-grade organic solvents (exposure to chlorophenols excluded) produced a relative risk of 11.2 (95% C.I. 3.2-39.7) based upon a few cases and controls with exposure to both. The authors concluded that "exposure to organic solvents, chlorophenols and/or phenoxy acids constitutes a risk factor for malignant lymphoma."

This latter study is still subject to the same methodological criticisms to which the earlier study was subjected. Chief among those is the possibility of observational and/or recall bias creeping into the responses that are elicited from self-administered questionnaires on kind and length of exposure. Secondly, confounding by exposure to potentially carcinogenic organic solvents and other agents could have had an effect, although the author assures the reader that they did not.

Other research has tentatively suggested that lumberjacks may be at increased risk of lymphoma (Edling and Granstam, 1979). The Nitro study found three deaths from cancers of the lymphatic and hematopoietic system, against only 0.88 expected (p=0.06, one-tailed Poisson test).

The lymphoma case-control study (Hardell et al., 1980, 1981) is consistent with the two STS studies discussed above. On the other hand, the consistency could also reflect an (as yet) unidentified common flaw in all these studies.

The two Swedish case control studies on STSs and a later case control study of malignant lymphoma (Hardell et al., 1981) were subjected to a validity analysis with respect to the assessment of exposure by Hardell and

Eriksson (1981). To answer the question raised regarding the recall of occupation in a forestry/agriculture job, secondary to the recall of exposure to phenoxy acids and/or chlorophenols, the cases and controls were divided into three groups: those who worked their entire time since 1950 in an agriculture/forestry job; those who worked some time in an agriculture/forestry job but not exclusively; and the remainder who never worked in a forestry/agriculture job. The study found that the risk ratio was still 8.2 for STS in exclusively agriculture/forestry workers who were exposed to phenoxy acids compared with workers found in other occupations having no apparent exposure to phenoxy acids or chlorophenols. Even when comparing phenoxy acid and/or chlorophenol exposed agricultural/forestry workers exclusively with nonexposed agricultural/forestry workers, the risk ratio was still 7.1. This argument seems to answer effectively questions regarding recall of occupation secondary to exposure.

On the other hand, the relative risk remains 5.4 when comparing phenoxy acid and/or chlorophenol exposed workers exclusively in occupations other than agriculture/forestry with nonexposed workers in those same occupations, thus, suggesting the presence of either recall bias or still another occupation with potential exposure to phenoxy acids and/or chlorophenols (Table 5-6).

When woodworkers are separated out (possible exposure to chlorophenols in treatment of wood) the risk ratio becomes 9.7 (Table 5-7). These data suggest the presence of some recall bias.

Another focus of this study was to determine if observational bias on the part of the investigators could explain the significantly high risk estimates. To answer the question, the study compared the exposure data

## TABLE 5-6

### Other Occupations (Minus Forestry/Agriculture)*

| Group | Phenoxy Acids/Chlorophenols | Non-exposed |
|---|---|---|
| Cases | 11 | 68 |
| Referents | 5 | 167 |
| | RR = 5.4 | $X^2$ = 11.01 ($p<0.01$) |

*Source: Hardell and Erikkson, 1981

## TABLE 5-7

Other Occupations (Minus Forestry/Agriculture/Woodworkers)*

| Group | Phenoxy Acids/Chlorophenols | Non-exposed |
|---|---|---|
| Cases | 4 | 66 |
| Referents | 1 | 160 |
|  | RR = 9.7 | $X^2$ = 5.98 ($p<0.05$) |

*Source: Hardell and Erikkson, 1981

derived from the interviewee's returned questionnaires only with the combined information from both the phone interviews and questionnaires. The study found no substantial differences in the frequency of reporting exposure.

Still a third consideration of possible bias involves recall of exposure to phenoxy acids and/or chlorophenols because of subject knowledge of having cancer in the cases versus no knowledge of cancer in the referent population. The study chose as a referent group for the 52 STS cases (Hardell and Sandstrom, 1979) and the 169 malignant lymphomas (Hardell et al., 1981) a group of 154 colon cancer cases from the same population source and compared their exposure to phenoxy acids and/or chlorophenols by broad age groupings, and by rural vs. urban residence.

Utilizing a Mantel-Haenszel rate ratio, the study found the risk of exposure to phenoxy acids remaining significantly high at 5.5 and to chlorophenols 5.4 in the STS cases compared with the colon cancer controls. Similarly, with the malignant lymphomas, the identically derived risk ratios remain significantly high at 4.5 with respect to phenoxy acids and/or chlorophenol exposure in the cases, hence, the study concludes, no "substantial observational bias" exists. If it is assumed in this study that recall bias was and is the same as observational bias, then such a conclusion may not be entirely warranted from the comparison. Certainly, it appears that no recall bias existed because of subject "knowledge of having cancer" based on the authors analysis. But it does not rule out the possibility that recall bias can still be present in their data for other reasons. Hardell refers to an intense "debate about phenoxy acids and their presumptive risk" in Sweden at the time the colon cancer study was conducted. But, there is no reason to think that colon cancer victims would assume their disease was brought about from exposure to dioxin containing chemicals if no connection was suggested.

It seems plausible that STS and/or non-Hodgkin's lymphoma patients would either learn at the time of their diagnosis that exposure to dioxin containing chemicals was the likely cause of this rare type of tumor or quickly learn from other sources, such as the news media, that exposure to herbicides containing dioxin could cause their rare form of cancer. Whereas, colon cancer victims (a rather common form of cancer) would not necessarily be led to believe that exposure to the same dioxin containing chemicals caused their disease. Hence, it is not difficult to imagine that such unusual victims of cancer could better "remember" exposure to such chemicals than could colon cancer patients.

Therefore, although this study may explain any biases introduced from secondary recall of occupation, observational bias introduced from the telephone interviewer and recall bias based on subject knowledge of cancer, it does not adequately answer questions of recall bias introduced through the acquired awareness on the part of the victim of STS or non-Hodgkin's lymphoma that his condition may have been caused by exposure to dioxin containing herbicides.

5.1.4. Stomach Cancer. Studies of two of the oldest cohorts of workers known to have been exposed to phenoxyacetic acid herbicides and/or 2,3,7,8-TCDD report stomach cancer mortality rates significantly higher than expected. The results in each study were based on small numbers of deaths. In one study (Axelson et al., 1980), 348 Swedish railroad workers with at least 46 days of herbicide exposure between 1955 and 1972 were followed through

October 1978. The workers were grouped on the basis of their primary herbicide exposures: those primarily exposed to phenoxyacetic acids (2,4-D and 2,4,5-T) only, to amitrole (aminotriazole) only, and to both types of herbicides. After a 10-year latency was achieved, 3 stomach cancer deaths were observed vs. 0.71 expected ($p<0.05$). None were attributable to amitrol alone, but two were assigned to phenoxy acids alone while the remaining stomach cancer death occurred in a worker exposed to both amitrol and phenoxy acids. The excess was more pronounced (3 observed vs. 0.57 expected, $p<0.05$) among those with early exposure (1957-1961) to phenoxy acids and/or amitrol. If persons who were exposed to just amitrol alone are excluded, thus leaving individuals exposed to phenoxy acid alone and amitrol in combination, the excess is enhanced further (3 observed vs. 0.41 expected, $p<0.01$).

Axelson et al. (1980) also notes an excess in total "tumors" after 10 years latency as well (15 observed vs. 6.87 expected, $p<0.005$). This is pronounced in those exposed early to phenoxy acids alone (6 observed vs. 2.60 expected, $p<0.01$) and phenoxy acids in combination with amitrol (5 observed vs. 1.34 expected, $p<0.05$). Presumably, "tumors" in Sweden are analogous to malignant neoplasms in the United States. The author states that no specific type of tumor predominates and no breakdown by tumor type is provided.

The other study showing increased stomach cancer mortality is the follow-up of 75 workers exposed to 2,3,7,8-TCDD during and after a 1953 runaway reaction at a trichlorophenol manufacturing facility in Ludwigshafen, Federal Republic of Germany (Thiess and Frentzel-Beyme, 1977). Two sources were used to calculate expected deaths: national mortality rates for the period 1971-1974, and 1972-1975 rates for Rhinehessen-Palatinate, the region in which Ludwigshafen is located.*

The results, shown in Table 5-8, indicate an increased rate of stomach cancer mortality that also is not likely to have been due to chance alone.

Two aspects of the methodology used could have influenced these results. First, the available report does not include an analysis allowing for a minimum period of cancer induction. All three stomach cancer deaths in the Ludwigshafen cohort occurred more than 10 years after initial exposure. Employing a 10-year restriction to follow-up (as in the Swedish cohort study) would result in a higher relative risk estimate by reducing the number of expected deaths.

Secondly, national and regional mortality rates from the 1970's were used to generate expected deaths to compare with observed mortality over a much longer period (1953-1977). The substantial decline in stomach cancer mortality in West Germany during the late 1950's and 1960's would likely make these expected figures too large.

The researchers also used an internal control group which does not raise the second concern discussed above. This group consisted of 75 men, each matched to study group members by age and date of entry into employment, and selected at random from a list of over 10,000 persons who had been included in previous cohort studies by the same investigators. No stomach cancer deaths occurred in this control group during the follow-up period. Thus, use of the internal control groups also indicates an excess of stomach cancers in the exposed workers.

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*The report originally included expected deaths using rates for the city of Ludwigshafen, which were later shown to be inaccurate.

## TABLE 5-8

### Analysis of Stomach Cancer Mortality in a Group of West German Factory Workers Exposed to 2,3,7,8-TCDD*

| Source for Expected Deaths | Stomach Cancer Deaths | | Relative Risk | Significance Level |
|---|---|---|---|---|
| | Observed | Expected | | |
| Federal Republic of Germany 1971-1974 | 3 | 0.559 | 5.4 | 0.02 |
| Rhinehessen-Palatinate 1972-1975 | 3 | 0.495 | 6.1 | 0.01 |

*Source: Thiess and Frentzel-Beyme, 1977

In an update of this earlier study, Thiess et al. (1982) continued the follow-up of his cohort through 1979 by adding 2 additional years of follow-up and apparently reducing the size of his cohort from 75 to 74. Altogether 21 deaths (4 more than from the earlier study) occurred vs. 18 and 19 deaths in the 2 matched (1 to 1) internal comparison groups. With respect to cancer deaths, the numbers were respectively 7, 5 and 5. The first control group was manually matched from the total number of persons (5500 included in the cohort until the end of 1976) and the second, at random, by computer for some 8000 employees. In addition, 19 expected total deaths were estimated based on 1970-1975 mortality statistics of Rhinehessin-Palatinate, 18 expected deaths based on 1970-1975 mortality statistics of Ludwigshafen, and 20 expected deaths based upon 1971-1974 mortality statistics of the Federal Republic of Germany. Just as in the earlier study, the three stomach carcinomas noted earlier appear to be significantly elevated regardless of which external comparison group is used (Table 5-9).

On the other hand, one stomach cancer appeared in the randomized internal control group. None appeared in the manually matched internal control. No other elevated risks for any other cause were evident and no STSs appeared. When latency was considered only, the risk of stomach cancer remained significantly elevated after a lapse of 10 years (3 observed, 0.52 expected, $p<0.016$) and then after a lapse of 15 years (2 observed, 0.23 expected, $p<0.02$) based upon death rates of Rhinehessin-Palatinate, 1970-1975.

Again, these study conclusions are limited by the small size of the study group and the very few cancer deaths noted at any particular site. Thus, it is insensitive to the detection of a significantly elevated risk for most causes of cancer, especially STS and lymphomas. Although, stomach cancer is elevated significantly, it is based only upon three deaths and

## TABLE 5-9

### Reanalysis of Stomach Cancer Mortality in a Group of West German Factory Workers Exposed to 2,3,7,8-TCDD*

| Source for Expected Deaths | Stomach Cancer Deaths | | Relative Risk | Significance Level |
|---|---|---|---|---|
| | Observed | Expected | | |
| Federal Republic of Germany 1971-1974 | 3 | 0.7 | 4.3 | 0.034 |
| Rhinehessin-Palatinate 1970-1975 | 3 | 0.64 | 4.7 | 0.027 |
| Ludwigs-Shafen 1970-1975 | 3 | 0.61 | 4.9 | 0.024 |

*Source: Thiess et al., 1982

since one stomach cancer death has been noted in an internal control group in the updated version, it appears that this finding has been weakened somewhat. Furthermore, as was pointed out earlier, trends in stomach cancer mortality during the 1950's, 1960's and 1970's could make the comparison of stomach cancer mortality with expected deaths less valid based upon 1970-1975 rates.

In summary, the evidence that phenoxyacetic acids and/or 2,3,7,8-TCDD might increase the risk of stomach cancer consists of two studies, each of which reports a statistically significant excess that is based on only three stomach cancer deaths. Further follow-up of these and similar cohorts is warranted, but firm conclusions cannot yet be made.

Four additional cohort studies have reported results that do not show increased stomach cancer mortality rates in groups of workers exposed to phenoxyacetic acids and/or 2,3,7,8-TCDD. These are studies of 2,4,5-T production workers in Midland, Michigan (Ott et al., 1980), Finnish phenoxyacetic acid herbicide applicators (Riihimaki et al., 1978), the Nitro study in which workers were exposed to 2,3,7,8-TCDD (Zack and Suskind, 1980) and trichlorophenol manufacturing workers (Cook et al., 1980).

As previously mentioned, the Nitro study included a single death from STS and a weakly suggestive increase in lymphatic and hematopoietic system cancer mortality. The Midland study included only one cancer death, a tumor in the respiratory system. In the Finnish study, histologic information on tumor types was not provided; however, there were no deaths from lymphoma.

The results pertinent to stomach cancer mortality in the three studies are shown in Table 5-10. Results of neither the Midland study nor the Nitro study contradict the findings of the Swedish and West German investigations previously discussed. This can be shown in two ways. First, the

## TABLE 5-10

### Stomach Cancer Mortality in Three Studies of Workers Exposed to Phenoxyacetic Acid Herbicides and/or 2,3,7,8-TCDD

| Stomach Cancer Deaths | | Relative Risk | 95% Confidence Interval | Reference |
|---|---|---|---|---|
| Observed | Expected | | | |
| 0 | 0.14[a] | 0 | 0-26.3 | Ott et al., 1980 |
| 5 | 6.9[a,b] | 0.7 | 0.2-1.7 | Riihimaki et al., 1978 |
| 0 | 0.5[b] | 0 | 0-7.4 | Zack and Suskind, 1980 |

[a] Estimated from total cancer expected deaths (see footnote in text).

[b] Entire follow-up period without regard for minimum time for cancer induction (Ott et al., 1980 used a 10-year minimum induction period).

upper 95% confidence limits for the relative risk estimates from these two "negative" studies exceed even the highest point estimates of relative risk (6.1) from the two "positive" studies (see Table 5-8).

This indicates that the relative risk estimates from the Midland and Nitro studies, even though equal to zero, are nevertheless not significantly different from the estimates of 6.1, given the sample sizes, follow-up periods, age distribution and comparison group rates.

In addition, the smallest detectable relative risk in the Midland study ($\alpha = 0.05$, $\varphi = 0.2$ one-tailed Poisson test) was 21.4 (3 observed deaths, 0.14 expected).* Similarly, the smallest detectable relative risk in the Nitro study ($\alpha = 0.05$, $\varphi = 0.2$, one-tailed Poisson test) was 10.0 (5 observed deaths, 0.5 expected). This calculation is based on results for the entire follow-up period. If, as in the Midland study, a minimum period of cancer induction had been employed, the expected deaths would have been fewer and the smallest reasonably detectable relative risk would have been greater. This analysis of statistical power indicates that the Nitro and Midland studies had very low probabilities of detecting the ~6-fold increases in risk suggested by the Swedish and West German investigations.

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*Ott et al. (1980) did not report expected deaths from stomach cancers. The figure 0.14 was obtained by multiplying the numbers of expected deaths from all cancers (2.6, allowing a 10-year minimum induction period) by the percentage of stomach cancers among the expected deaths in the Nitro study (0.5/9.04 = 5.5%). The two studies used United States white male mortality rates and covered similar calendar years in follow-up (1949-1978 in Nitro and 1950-1976 in Midland), but a similarity in age distributions cannot be established from the published reports.

Statistically, the study of Finnish herbicide applicators is inconsistent with the results of the Swedish and West German cohort studies. The smallest reasonably detectable relative risk ($\alpha = 0.05$, $\varphi = 0.2$, one-tailed Poisson test) was only 3.1 (11 observed deaths, 3.6 expected).* The study, therefore, appears powerful enough to detect relative risks even smaller than those seen in the Swedish and West German studies. A partial explanation for this apparent inconsistency could lie in the fact that the Finnish study set the minimum period of herbicide exposure for membership in the cohort at 10 days (2 working weeks) and noted that the "total strength of exposure has, in most cases, been a few weeks only." The Swedish study of herbicide applicators set the minimum exposure at 46 days (>1 spraying season).

There are also certain inconsistencies in the data from the Finnish study which the authors note but find difficult to explain. In particular, no cancer deaths occurred during the latter part of the study period among Forestry Authority workers (1 of 4 groups included in the cohort), even though 9.0 deaths were expected. This finding strongly suggests some deficiency in follow-up or in the source records from which vital status was determined.

In summary, four cohort studies of workers exposed to phenoxyacetic acid herbicides and/or 2,3,7,8-TCDD do not report increased risks of stomach cancer. Only one of these, however, was statistically powerful enough to be inconsistent with the two studies that tentatively suggest an increase in stomach cancer risk. The available report of this study of Finnish herbicide applicators contains methodologic questions that require clarification.

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*The expected stomach cancer deaths were estimated in the same manner as for the Midland study. A proportion of 20% of all cancer deaths was applied because Finnish male mortality rates are known to be very high.

**5.1.5. Animal Studies.** When outbred Swiss mice were given weekly doses of TCDD by gavage, an increase in liver tumors was observed (Toth et al., 1979). Animals receiving 0.007 µg/kg/week for 1 year showed an elevated tumor incidence over vehicle-treated mice; in the 0.7 µg/kg/week group the increase was statistically significant ($p<0.005$). Mortality was sufficiently high at 7.0 µg/kg/week as to interfere with carcinogenicity evaluation.

DiGiovanni et al. (1977) reported a mouse skin painting study. The authors indicated that TCDD was a weak initiator on the skin.

A bioassay of 2,3,7,8-TCDD for possible carcinogenicity was conducted by Illinois Institute of Technology Research, Chicago, Illinois, on a contract with the NCI Carcinogenesis Testing Program by dermal administration of the test material in Swiss-Webster mice for 104 weeks (U.S. DHHS, 1980a). Thirty male and female Swiss-Webster mice were dermally treated with an acetone suspension of TCDD for 3 days/week for 104 weeks. Similar groups were pretreated with 1 application of 50 µg dimethylbenzanthracene (DMBA) in 0.1 mℓ acetone 1 week before TCDD administration began. Female mice received 0.005 µg TCDD per application, and the male mice received 0.001 µg TCDD. As vehicle controls, 45 mice of each sex received 0.1 mℓ acetone 3 times/week. Thirty animals of each sex were used as untreated controls (Tables 5-11 and 5-12).

Throughout the bioassay, mean body weights of the male or female groups of mice administered TCDD, or TCDD following DMBA, were essentially the same as those of the corresponding vehicle-control group. Mean body weights of dosed and vehicle-control groups of the females were less than those of the untreated control group throughout the study, and for the males were less than mean body weights of untreated controls during the first 80 weeks.

## TABLE 5-11

## Incidence of Primary Tumors in Female Swiss-Webster Mice by Dermal Application of 2,3,7,8-TCDD or 2,3,7,8-TCDD Following DMBA[a]

| Tissue: Types of Neoplastic Growth | Vehicle Control | 2,3,7,8-TCDD | 2,3,7,8-TCDD plus DMBA |
|---|---|---|---|
| Integumentary System: | | | |
| Fibrosarcoma | 2/41 (5%) | 8/27 (30%)[b] | 8/29 (28%)[c] |
| Lung: | | | |
| Alveolar/Bronchiolar Adenoma | 4/41 (10%) | 1/25 (4%) | 3/28 (11%) |
| Alveolar/Bronchiolar Carcinoma | 5/41 (12%) | 1/25 (4%) | 3/28 (11%) |
| Alveolar/Bronchiolar Carcinoma or Adenoma | 9/41 (22%) | 2/25 (8%) | 6/28 (21%) |
| Hematopoietic System: | | | |
| Lymphoma | 14/41 (34%) | 10/27 (37%) | 8/29 (28%) |
| All Sites: | | | |
| Hemangioma | 2/41 (5%) | 0/27 (0%) | 1/29 (3%) |
| Hemangioma or Hemangiosarcoma | 3/41 (7%) | 0/27 (0%) | 1/29 (3%) |

[a] Source: U.S. DHHS, 1980a

[b] $p<0.007$

[c] $p<0.010$

## TABLE 5-12

### Incidence of Primary Tumors in Male Swiss-Webster Mice by Dermal Application of 2,3,7,8-TCDD or 2,3,7,8-TCDD Following DMBA*

| Tissue: Types of Neoplastic Growth | Vehicle Control | 2,3,7,8-TCDD | 2,3,7,8-TCDD plus DMBA |
|---|---|---|---|
| Integumentary System: | | | |
| Fibrosarcoma | 3/42 (7%) | 6/28 (21%) | 6/30 (20%) |
| Lung: | | | |
| Alveolar/Bronchiolar Adenoma | 6/41 (15%) | 1/28 (4%) | 5/29 (17%) |
| Alveolar/Bronchiolar Carcinoma | 1/41 (2%) | 1/28 (4%) | 2/29 (7%) |
| Alveolar/Bronchiolar Carcinoma or Adenoma | 7/41 (17%) | 2/28 (7%) | 6/29 (21%) |
| Hematopoietic System: | | | |
| Lymphoma or Leukemia | 4/42 (10%) | 2/28 (7%) | 5/30 (17%) |
| All Sites: | | | |
| Hemangiosarcoma | 1/42 (2%) | 4/28 (14%) | 0/30 (0%) |

*Source: U.S. DHHS, 1980a

In female mice, the incidence of fibrosarcoma in the integumentary system in groups dosed with TCDD and TCDD following DMBA was significantly higher than that in the corresponding controls (see Table 5-11).

In was concluded that, under the conditions of this bioassay, TCDD applied to the skin was carcinogenic for female Swiss-Webster mice, inducing increased incidences of fibrosarcoma in the integumentary system (U.S. DHHS, 1980a).

Van Miller et al. (1977a,b) administered 0, 1, 5, 50, 1000 and 5000 ppt of TCDD in the diet of male Sprague-Dawley rats. Higher concentrations (50, 500 and 1000 ppb) were also administered, but all of the rats fed at those three highest concentrations died early in the test. After 65 weeks, all surviving animals underwent laparotomies, and all tumors were biopsied. The rats were kept on the TCDD diet for a total of 78 weeks and then placed on the control diet. After a total of 95 weeks all surviving animals were sacrificed and necropsied. The results of the study are summarized in Table 5-13.

Although the study by Van Miller et al. (1977a,b) demonstrated the occurrence of neoplasms upon TCDD exposure, the study has a number of shortcomings. The protocol was unusual and only a small number of animals were used. The occurrence of tumors did not follow a clear-cut dose-response relationship. Furthermore, the complete absence of tumors in the controls is a highly unusual finding.

Kociba et al. (1978) reported a more extensive carcinogenicity study on TCDD. Groups of 100 Sprague-Dawley rats (Spartan substrain: 50 males and 50 females per group) were maintained for up to 2 years on diets supplying 0.1, 0.01 or 0.001 μg TCDD/kg/day. The control group consisted of 86 males and 85 females. The terminal necrospy examination was conducted at the end of 2

## TABLE 5-13

Summary of Neoplastic Changes After 2,3,7,8-TCDD in Rats[a]

| Concentration of 2,3,7,8-TCDD in Diet (ppt) | Approximate Daily Dose (μg/kg) | No. of Animals with Neoplasms[b] | No. of Neoplasms | Diagnosis |
|---|---|---|---|---|
| 0 | 0.0 | 0 | 0 | NA |
| 1 | 0.00004 | 0 | 0 | NA |
| 5 | 0.0001 | 5 | 6 | 1 ear duct carcinoma |
| | | | | 1 lymphocytic leukemia |
| | | | | 1 adenocarcinoma (kidney) |
| | | | | 1 malignant histiocytoma (peritoneal) |
| | | | | 1 angiosarcoma (skin) |
| | | | | 1 Leydig cell adenoma |
| 50 | 0.0014 | 3 | 3 | 1 fibrosarcoma (muscle) |
| | | | | 1 squamous cell tumor (skin) |
| | | | | 1 astrocytoma (brain) |
| 500 | 0.014 | 4 | 4 | 1 fibroma (muscle) |
| | | | | 1 carcinoma (skin) |
| | | | | 1 adenocarcinoma (kidney) |
| | | | | 1 sclerosing seminoma (testes) |
| 1000 | 0.057 | 4 | 5 | 1 cholangiocarcinoma (liver) |
| | | | | 1 angiosarcoma (skin) |
| | | | | 1 glioblastoma (brain) |
| | | | | 2 malignant histiocytoma (peritoneal) |
| 5000 | 0.29 | 7 | 10 | 4 squamous cell tumors (lung) |
| | | | | 4 neoplastic nodules (liver) |
| | | | | 2 cholangiocarcinomas (liver) |

[a] Source: Van Miller et al., 1977a

[b] 10 animals per group

NA = Not applicable

years of treatment. Females recieving 0.1 μg TCDD/kg/day experienced a greater mortality than controls during the second half of the study. Extensive clinical chemistry data were reported as part of the study.

A portion of the data for the histopathologic lesions found is summarized in Table 5-14. The only lesions that are listed are those which were statistically different from control levels for at least one dose and in one sex. The following neoplastic lesions were found to be increased above control levels ($p<0.05$):

Hepatocellular hyperplastic (neoplastic) nodules - females only

Hepatocellular carcinomas - females only

Stratified squamous cell carcinoma of palate or nasal turbinae - males and females

Keratinizing squamous carcinoma of the lung - females only

Stratified squamous carcinoma of the tongue - males only

Adrenal cortical ademona - males only

Dr. Robert Squire, pathologist at the Johns Hopkins University Medical School and consultant to the U.S. EPA Carcinogens Assessment Group, evaluated the histopathologic slides from the 2-year rat feeding studies on 2,3,7,8-TCDD by Kociba et al. (1978). Squire and his associates examined all livers, tongues, hard palates and nasal turbinates, and lungs available from the TCDD study. His histopathological findings, as well as Kociba's histopathological evaluations, are summarized in Tables 5-15 and 5-16. Although there are some differences between the diagnoses of Kociba and Squire, the conclusions about the target organ for cancer induction and the dose levels at which induction occurred are the same whether Squire's or Kociba's diagnoses are considered.

# TABLE 5-14

## Summary of Neoplastic Lesions Produced by 2,3,7,8-TCDD in Sprague-Dawley Rats, Spartan Substrain that are Statistically Significant in at Least One Sex[a]

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Dose μg/kg/day | 0 | 0.001 | 0.01 | 0.1 | 0 | 0.001 | 0.01 | 0.1 |
| Number of Animals | 85 | 50 | 50 | 50 | 86 | 50 | 50 | 50 |
| Hepatocellular hyperplastic nodule | 6 | 0 | 2 | 3 | 8 | 3 | 18[b] | 23[b] |
| Hepatocellular carcinomas | 2 | 0 | 0 | 1 | 1 | 0 | 2 | 11[b] |
| Stratified squamous carcinoma-palate (including nasal turbinate tumors) | 0 | 0 | 0 | 4[b] | 0 | 0 | 1 | 4[b] |
| Lung-keratinizing squamous carcinoma | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 7[b] |
| Benign tumor-uterus | - | - | - | - | 28 | 12 | 11 | 7[b] |
| Subcutaneous fibroma/fibroadenoma/lipoma | 10 | 1[b] | 5 | 6 | 1 | 1 | 0 | 0 |
| Benign mammary neoplasm | 0 | 0 | 1 | 2 | 73 | 35 | 36 | 24[b] |
| Mammary carcinoma | 0 | 0 | 0 | 2 | 8 | 4 | 4 | 0 |
| Stratified squamous carcinoma-tongue | 0 | 1 | 1 | 3[b] | 1 | 0 | 0 | 2 |
| Pituitary adenoma | 26 | 6 | 11 | 13 | 43 | 18 | 13 | 12[b] |
| Acinar adenoma pancreas | 14 | 7 | 5 | 2[b] | 0 | 1 | 0 | 1 |
| Adenoma-adrenal cortex | 0 | 0 | 2 | 5[b] | 9 | 6 | 2 | 5 |
| Pheochromocytoma | 28 | 6 | 10 | 4[b] | 7 | 2 | 1 | 3 |

[a] Source: Kociba et al., 1978

[b] Significantly different ($p<0.05$) from control by the Fisher exact text

## TABLE 5-15

2,3,7,8-TCDD Oral Rat Study by Dr. Kociba, with Dr. Squire's Review (8/15/80)
Female Sprague-Dawley Rats - Spartan Substrain (2 years)

| Tissues and Diagnoses* | Dose Levels (μg/kg/day) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 (control) | | 0.001 | | 0.01 | | 0.1 | |
| | S | K | S | K | S | K | S | K |
| Lung squamous cell carcinomas | 0/86 | 0/86 | 0/50 | 0/50 | 0/49 | 0/49 | 8/47<br>($P<10^{-3}$) | 7/49<br>($P<10^{-3}$) |
| Nasal turbinate/hard palate squamous cell carcinomas | 0/54 | 1/54 | 0/30 | 0/30 | 1/27 | 1/27 | 5/22<br>($P<10^{-2}$) | 5/24<br>($P<10^{-2}$) |
| Liver Neoplastic nodules/hepatocellular carcinomas | 16/86 | 9/86 | 8/50 | 3/50 | 27/50<br>($P<10^{-4}$) | 18/50<br>($P<10^{-3}$) | 33/47<br>($P<10^{-8}$) | 34/48<br>($P<10^{-12}$) |
| Total combined (1,2, or above) (each animal had at least one tumor above) | 16/86 | 9/86 | 8/50 | 3/50 | 27/50<br>($P<10^{-4}$) | 18/50<br>($P<10^{-3}$) | 34/47<br>($P<10^{-8}$) | 34/49<br>($P<10^{-11}$) |

*Where result is significantly different than the appropriate control, probability (P) of incorrectly rejecting the null hypothesis is approximated in parentheses.

S = Dr. Squire's histopathologic analysis; K = Dr. Kociba's histopathologic analysis

## TABLE 5-16

### 2,3,7,8-TCDD Oral Rat Study by Dr. Kociba, with Dr. Squire's Review (8/15/80)
### Male Sprague-Dawley Rats - Spartan Substrain (2 years)

| Tissues and Diagnoses* | Dose Levels (µg/kg/day) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 (control) | | 0.001 | | 0.01 | | 0.1 | |
| | S | K | S | K | S | K | S | K |
| Nasal turbinate/ hard palate squamous cell carcinomas | 0/55 | 0/51 | 1/34 | 1/34 | 0/26 | 0/27 | 6/30<br>($P<10^{-2}$) | 4/30 |
| Tongue squamous cell carcinomas | 0/77 | 0/76 | 2/44 | 1/49 | 1/49 | 1/49 | 3/44<br>($P<0.05$) | 3/42<br>($P<10^{-2}$) |
| Total - 1 or 2 above (each rat had at least one tumor above) | 0/77 | 0/76 | 2/44 | 2/49 | 1/49 | 1/49 | 9/44<br>($P<10^{-4}$) | 7/42 |

*Where result is significantly different than the appropriate control, probability (P) of incorrectly rejecting the null hypothesis is approximated in parentheses.

S = Dr. Squire's histopathologic analysis; K = Dr. Kociba's histopathologic analysis

A bioassay of 2,3,7,8-TCDD, for possible carcinogenicity was conducted by administering the test material by gavage to Osborne-Mendel rats and B6C3F$_1$ mice for 104 weeks (U.S. DHHS, 1980b). Fifty rats and mice of each sex were administered TCDD suspended in a vehicle of 9:1 corn oil-acetone 2 days/week for 104 weeks at doses of 0.01, 0.05 or 0.5 µg/kg/week for rats and male mice and 0.04, 0.2 or 2.0 µg/kg/week for female mice. Seventy-five rats and 75 mice of each sex served as vehicle controls. One untreated control group containing 25 rats and 25 mice of each sex was present in the TCDD treatment room, and one untreated control group containing 25 rats and 25 mice of each sex was present in the vehicle control room. All surviving animals were killed at 105-107 weeks.

In rats, a dose-related depression in mean body weight gain became evident in the males after week 55 of the bioassay and in the females after week 45. In mice, the mean body weight gain in the dosed groups was comparable with that of the vehicle-control groups, but it was lower than that of the untreated controls.

In male rats, increased incidences of follicular-cell adenoma or carcinomas in the thyroid were dose related and were significantly higher in the low-, mid- and high-dose groups (p=0.001 for high dose) than in the vehicles controls (Table 5-17). A significant increase in the subcutaneous tissue fibroma was found for the high-dose group (p=0.048).

In female rats, the incidences of follicular-cell adenomas of the thyroid and hepatocellular carcinomas or neoplastic nodules of the liver were dose-related, and the incidence of hepatocellular carcinomas in the high-dose group was significantly higher (p=0.001) than that in the vehicle controls (Table 5-18), as were the incidences of subcutaneous tissue fibrosarcoma (p=0.023) and adrenal cortical adenoma (p=0.039).

## TABLE 5-17

## Incidence of Primary Tumors in Male Osborne-Mendel Rats (2,3,7,8-TCDD Administered by Gavage)[a,b]

| Tissue: Types of Neoplastic Growth | Vehicle Control | Dose (μg/kg/wk)[c] | | |
|---|---|---|---|---|
| | | 0.01 | 0.05 | 0.5 |
| Subcutaneous Tissue: | | | | |
| Fibroma or Fibrosarcoma | 12/75(16) | 4/50(8) | 5/50(10) | 10/50(20) |
| Fibroma | 3/75(4) | 1/50(2) | 3/50(6) | 7/50(14)[d] |
| Fibrosarcoma | 9/75(12) | 3/50(6) | 3/50(6) | 3/50(6) |
| Circulatory System: | | | | |
| Hemangioma or Hemangiosarcoma | 7/75(9) | 3/50(6) | 1/50(2) | 4/50(8) |
| Hemangiosarcoma | 4/75(5) | 3/50(6) | 0/50(0) | 4/50(8) |
| Liver: | | | | |
| Neoplastic Nodule or Hepatocellular Carcinoma | 0/74(0) | 0/50(0) | 0/50(0) | 3/50(6) |
| Neoplastic nodule | 0/74(0) | 0/50(0) | 0/50(0) | 3/50(6) |
| Pituitary: | | | | |
| Adenoma or Chromophobe Adenoma | 2/61(3) | 1/43(2) | 3/43(7) | 3/40(8) |
| Adenoma | 0/61(0) | 1/43(2) | 2/43(5) | 3/40(8) |
| Adrenal: | | | | |
| Cortical Adenoma | 6/72(8) | 9/50(18) | 12/49(24) | 9/49(18) |
| Pheochromcytoma | 5/72(7) | 0/50(0) | 1/49(2) | 1/49(2) |
| Thyroid: | | | | |
| Follicular Cell Adenoma or Carcinoma | 1/69(1) | 5/48(10)[e] | 8/50(16)[f] | 11/50(22)[g] |
| Follicular Cell Adenoma | 1/69(1) | 5/48(10) | 6/50(12) | 10/50(20) |
| C-Cell Adenoma | 2/69(3) | 2/48(4) | 4/50(8) | 4/50(8) |
| C-Cell Adenoma or Carcinoma | 2/69(3) | 2/48(4) | 5/50(10) | 4/50(8) |
| Parathyroid: | | | | |
| Adenoma | 0/20(0) | 2/41(5) | 1/40(3) | 1/36(3) |
| Pancreatic Islets: | | | | |
| Adenoma | 2/70(3) | 2/49(4) | 3/48(6) | 1/50(2) |

TABLE 5-17 (cont.)

| Tissue: Types of Neoplastic Growth | Vehicle Control | Dose (μg/kg/wk)[c] | | |
|---|---|---|---|---|
| | | 0.01 | 0.05 | 0.5 |
| Mammary Gland: | | | | |
| Adenocarcinoma | 0/75(0) | 0/50(0) | 3/50(6) | 1/50(2) |
| Fibroadenoma | 5/75(7) | 0/50(0) | 1/50(2) | 0/50(0) |

[a] Source: U.S. DHHS, 1980b

[b] Values in parentheses indicate percent response.

[c] $p$ = Values calculated using the Fisher Exact test.

[d] $p = 0.048$

[e] $p = 0.042$

[f] $p = 0.004$

[g] $p < 0.001$

## TABLE 5-18

### Primary Tumors in Female Osborne-Mendel Rats (2,3,7,8-TCDD Administered by Gavage)[a,b]

| Tissue: Types of Neoplastic Growth | Vehicle Control | Dose (μg/kg/wk)[c] | | |
|---|---|---|---|---|
| | | 0.01 | 0.05 | 0.5 |
| Subcutaneous Tissue: | | | | |
| Fibroma or Fibrosarcoma | 4/75(5) | 2/50(4) | 3/50(6) | 5/49(10) |
| Fibroma | 4/75(5) | 0/50(0) | 0/50(0) | 1/49(2) |
| Fibrosarcoma | 0/75(0) | 2/50(4) | 3/50(6) | 4/49(8)[d] |
| Liver: | | | | |
| Neoplastic Nodule or Hepatocellular Carcinoma | 5/75(7) | 1/49(2) | 3/50(6) | 14/49(29)[e] |
| Neoplastic nodule | 5/75(7) | 1/49(2) | 3/50(6) | 12/49(24) |
| Pituitary: | | | | |
| Adenoma | 1/66(2) | 5/47(11) | 2/44(5) | 3/43(7) |
| Chromophobe Adenoma | 5/66(8) | 0/47(0) | 0/44(0) | 1/43(2) |
| Adrenal: | | | | |
| Cortical Adenoma or Adenoma | 11/73(15) | 8/49(16) | 4/49(8) | 14/46(30)[f] |
| Cortical Adenoma or Carcinoma or Adenoma | 11/73(15) | 9/49(18) | 5/49(10) | 14/46(30) |
| Thyroid: | | | | |
| Follicular Cell Adenoma | 3/73(4) | 2/45(4) | 1/49(2) | 6/47(13) |
| Follicular Cell Adenoma or Carcinoma | 5/73(7) | 2/45(4) | 1/49(2) | 6/47(13) |
| C-Cell Adenoma | 7/73(10) | 1/45(2) | 8/49(26) | 6/47(13) |
| C-Cell Adenoma or Carcinoma | 7/73(10) | 3/45(7) | 8/49(16) | 6/47(13) |
| Mammary Gland: | | | | |
| Adenocarcinoma | 3/75(4) | 3/50(6) | 2/50(4) | 1/49(2) |
| Fibroadenoma | 27/75(36) | 20/50(40) | 21/50(42) | 17/49(35) |
| Brain: | | | | |
| Astrocytoma | 0/75(0) | 3/47(6) | 0/49(0) | 0/48(0) |

---

[a] Source: U.S. DHHS, 1980b

[b] Values in parentheses indicate percent response.

[c] P = Values calculated using the Fisher Exact test.

[d] P = 0.0023; [e] P = 0.001; [f] P = 0.039

In both male and female mice, incidences of hepatocellular adenomas or carcinomas were dose related and the incidences in the high-dose groups were higher ($p=0.001$ male, $p=0.002$ female) than those in the corresponding vehicle controls (Tables 5-19 and 5-20).

In female mice, follicular-cell adenomas in the thyroid and histocytic lymphomas in the hematopoietic system occurred at dose-related incidences, and the incidences were significantly higher in the high-dose groups than those in vehicle controls. The high-dose group of females also showed a significantly higher incidence of subcutaneous fibrosarcomas ($p=0.032$) and lymphoma or leukemia ($p=0.029$) (see Table 5-20).

It was concluded that, under the conditions of this bioassay, TCDD was carcinogenic for Osborne-Mendel rats, inducing significant dose-related increased incidences of follicular-cell thyroid tumors in males and liver tumors in females. TCDD was also carcinogenic for B6C3F$_1$ mice, inducing significant dose-related increased incidences of liver tumors in males and females and of thyroid tumors and fibrosarcoma of subcutaneous tissue in females (U.S. DHHS, 1980b).

2,3,7,8-TCDD is probably carcinogenic for humans on the basis of animal carcinogenicity studies which were positive in multiple species and organs. Epidemiological studies of workers exposed to chemicals contaminated with TCDD such as 2,4,5-trichlorophenoxyacetic acid and 2,4,5-trichlorophenol are consistent with the position that TCDD is probably carcinogenic for humans; the available evidence indicates an excess incidence of STSs. Because TCDD is almost always found in association with other materials (e.g., chlorophenols, combustion products, etc.), it may never be possible to evaluate the carcinogenicity of TCDD by itself in humans.

## TABLE 5-19

## Incidence of Primary Tumors in Female B6CF1 Mice (2,3,7,8-TCDD Administered by Gavage)[a,b]

| Tissue: Types of Neoplastic Growth | Vehicle Control | Dose (µg/kg/wk)[c] | | |
|---|---|---|---|---|
| | | 0.01 | 0.05 | 0.5 |
| Subcutaneous Tissue: | | | | |
| Fibrosarcoma | 1/74(1) | 1/50(2) | 1/48(2) | 5/47(11)[d] |
| Lung: | | | | |
| Alveolar/Bronchiolar Adenoma | 2/74(3) | 3/49(6) | 4/48(8) | 1/46(2) |
| Alveolar/Bronchiolar Adenoma or Carcinoma | 2/74(3) | 3/49(8) | 4/48(8) | 2/46(4) |
| Hematopoietic System: | | | | |
| Lymphocytic Lymphoma | 5/74(7) | 6/50(12) | 4/48(8) | 6/47(13) |
| Histiocytic Lymphoma | 9/74(12) | 4/50(8) | 8/48(17) | 14/47(30)[e] |
| All Lymphoma | 18/74(24) | 11/50(22) | 13/48(27) | 20/47(43)[f] |
| Lymphoma or Leukemia | 18/74(24) | 12/50(24) | 13/48(27) | 20/47(43)[f] |
| Liver: | | | | |
| Hepatocellular Adenoma or Carcinoma | 3/73(4) | 6/50(12) | 6/48(13) | 11/47(23)[g] |
| Hepatocellular Adenoma | 2/73(3) | 4/50(8) | 4/48(8) | 5/47(11) |
| Hepatocellular Carcinoma | 1/73(1) | 2/50(4) | 2/48(4) | 6/47(13) |
| Pituitary: | | | | |
| Adenoma | 0/62(0) | 2/39(5) | 0/38(0) | 2/33(6) |
| Thyroid: | | | | |
| Follicular-Cell Adenoma | 0/69(0) | 3/50(6) | 1/47(2) | 5/46(11)[h] |

[a] Source: U.S. DHHS, 1980b

[b] Values in parentheses indicate percent response.

[c] p = Values calculated using the Fisher Exact test.

[d] p = 0.032; [e] p = 0.016; [f] p = 0.029; [g] p = 0.002; [h] p = 0.009

## TABLE 5-20

## Incidence of Primary Tumors in Male B6CF1 Mice (2,3,7,8-TCDD Administered by Gavage)[a,b]

| Tissue: Types of Neoplastic Growth | Vehicle Control | Dose (μg/kg/wk)[c] | | |
|---|---|---|---|---|
| | | 0.01 | 0.05 | 0.5 |
| Subcutaneous Tissue: | | | | |
| Fibrosarcoma or Fibroma | 9/73(12) | 6/49(12) | 5/49(10) | 3/50(6) |
| Fibrosarcoma | 8/73(11) | 5/49(10) | 4/49(8) | 3/50(6) |
| Lung: | | | | |
| Alveolar/Bronchiolar Adenoma or Carcinoma | 10/71(14) | 2/48(4) | 4/48(8) | 13/50(26) |
| Alveolar/Bronchiolar Adenoma | 7/71(10) | 2/48(4) | 4/48(8) | 11/50(22) |
| Hematopoietic System: | | | | |
| Histiocytic Lymphoma | 5/73(7) | 0/49(0) | 3/49(6) | 0/50(0) |
| Lymphoma or Leukemia | 8/73(11) | 3/49(6) | 4/49(8) | 6/50(12) |
| Circulatory System: | | | | |
| Hemangiosarcoma | 1/73(1) | 2/49(4) | 1/49(2) | 3/50(6) |
| Liver: | | | | |
| Hepatocellular Adenoma or Carcinoma | 15/73(21) | 12/49(24) | 13/49(27) | 27/50(54)[d] |
| Hepatocellular Adenoma | 7/73(10) | 3/49(6) | 5/49(10) | 10/50(20) |
| Hepatocellular Carcinoma | 8/73(11) | 9/49(18) | 8/49(16) | 17/50(34) |
| Thyroid: | | | | |
| Follicular-Cell Adenoma | 0/69(0) | 3/48(6) | 0/48(0) | 0/49(0) |
| Eye/Lacrimal Glands: | | | | |
| Adenoma | 0/73(0) | 1/49(2) | 1/49(2) | 3/50(6) |

[a] Source: U.S. DHHS, 1980b

[b] Values in parentheses indicate percent response.

[c] p = Values calculated using the Fisher Exact test.

[d] $p<0.001$

No animal carcinogenicity studies on PeCDDs were located in the available literature.

Hexachlorodibenzo-p-dioxin has also been tested for carcinogenicity in rats and mice treated by gavage (U.S. DHHS, 1980c) and by dermal application to mice (U.S. DHHS, 1980d). In these studies, a 1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HCDD was tested. In the oral study (U.S. DHHS, 1980c), animals received HCDD at doses of 0.0, 1.25, 2.5 or 5.0 μg/kg/week, except for female mice which received 0.0, 2.5, 5.0 and 10.0 μg/kg/week. The following impurities were detected in $H_xCDD$ used in this bioassay: PeCDD, <0.004%; TCDD, 0.09% (±0.03%), TriCDD, 0.004%; DCDD, <0.004%; and Bromo-PeCDD, <0.004%. The specific isomers of these impurities were not identified. In both species and both sexes, only tumors of the liver occurred at a significantly greater incidence than in controls. In male rats and male and female mice, the liver tumor incidence was significantly increased over control values only in the high-dose groups, while in female rats the incidence was significantly greater at both the medium and high dose levels. In the study of HCDD carcinogenicity in mouse skin conducted by NTP (U.S. DHHS, 1980d), there were no treatment-related tumors in either the carcinogenicity bioassay or the tumor promotion assay using DMBA as an initiator.

Reexamination of microscopic slides from NTP's $H_xCDD$ bioassay (Squire Assoc., Inc., 1983; Tracor Jitco, Inc., 1983; U.S. EPA, 1984a) revealed that many hepatic lesions diagnosed as neoplastic by NTP were in reality non-neoplastic. Squire Assoc., Inc. (1983) concluded that the results from the NTP bioassay provided only equivocal evidence of a weak hepatocarcinogenic effect in female rats and mice, and only at levels which are severely hepatotoxic. However, U.S. EPA (1984a) and Tracor Jitco, Inc. (1983) concurred independently with the conclusion that 1,2,3,6,7,8- and 1,2,3,7,8,9-$H_xCDD$

mixture was carcinogenic, indicated by a statistically significant increased incidence of hepatic tumors in female rats and both sexes of mice, and by a borderline hepatocarcinogenic response in male rats.

5.1.6. Conclusion. Because of the induction of hepatocellular carcinoma in two strains of female rats and both sexes of one mouse strain, along with the induction of thyroid tumors, subcutaneous fibrosarcomas, and lung and tongue tumors in both rats and mice, the evidence of carcinogenicity for TCDD in animals would be regarded as sufficient if the IARC classification system were used. These effects occur at extremely low doses. The demonstration of a promotion effect in rat liver after initiation with diethyl nitrosamine, and a cocarcinogenic response when TCDD was injected simultaneously with 3-methyl chloranthrene increases the level of evidence in animals.

However, with respect to the dioxin impurities contained in these herbicides, the evidence for the human carcinogenicity for 2,3,7,8-TCDD, based on the epidemiologic studies, is only suggestive because of the difficulty of evaluating the risk of 2,3,7,8-TCDD exposure in the presence of the confounding effects of phenoxy acids and/or chlorophenol. Therefore, the overall evidence of carcinogenicity, considering both animal and human studies, would place 2,3,7,8-TCDD alone in the 2B category of the International Agency for Research on Cancer (IARC), and 2,3,7,8-TCDD in association with the phenoxy herbicides and/or chlorophenols in the 2A category. The IARC regards chemicals in both categories as a probable carcinogen in humans.

Hepatocellular tumors have been induced in mice and rats following administration of a 1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HCDD. This level of carcinogenic evidence in animals would be regarded as "sufficient" according to the IARC classification scheme. Therefore, based on animal evidence, HCDD would be placed in Group 2B, which IARC characterizes as probably carcinogenic in humans.

## 5.2. MUTAGENICITY

Short term in vitro test systems have been developed to assess the biologic, toxic and genotoxic effects of chemicals. These assays have proven to be useful indicators of potential activity of diverse industrial chemicals, a broad range of drugs and xenobiotics, carcinogens and crude environmental extracts. The most widely used short-term test system, the Ames test for bacterial mutagenesis, employs several strains of Salmonella typhimurium that are highly susceptible to the effects of mutagenic chemicals. Despite the obvious utility of the Ames test and related short-term assays, their predictive capabilities (i.e., the correlation between bacterial mutagenicity and carcinogenicity) have not been fully assessed (Bartsch et al., 1982).

Mutagenicity assays in microorganisms have been used to assess the genotoxic effects of 2,3,7,8-TCDD; however, the results of most of these assays have indicated little potential for mutagenic effects (Table 5-21).

Hussain et al. (1972) exposed Salmonella typhimurium histidine-dependent strains TA1530 and TA1532 in liquid suspension to 2,3,7,8-TCDD followed by plating into selective medium to observe reversion to prototypes. No increase in the reversion rate was observed with strain TA1530 at exposure levels of 1 and 10 μg/mℓ. These exposures resulted in cell survivals of 90 and <1%, respectively. In strain TA1532, increased reversion frequency was not observed at 2,3,7,8-TCDD concentrations of 2-3 μg/mℓ, which resulted in a 0-50% decrease in survival; however, at 2,3,7,8-TCDD levels that resulted in a 99% decrease in survival, there was an increased number of revertant colonies/surviving cells. The dose levels were not specified.

### TABLE 5-21

### The Results of Mutagenicity Assays for 2,3,7,8-TCDD in *Salmonella typhimurium*

| Type of Assay | Strains of *Salmonella typhimurium* | | | | | | | | | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S-9 | TA98 | TA1530 | TA1535 | TA1537 | TA1538 | TA1532 | TA1950 | TA1975 | TA1978 | G46 | TA100 | TA1531 | TA1534 | |
| Spot test | +/- | NT | NT | 0 | 0 | 0 | 0 | NT | NT | NT | NT | NT | NT | NT | McCann, 1978 |
| Plate incorporation | +/- | NT | NT | 0 | 0 | 0 | 0 | NT | NT | NT | NT | NT | NT | NT | McCann, 1978 |
| Plate incorporation* | +/- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | NT | NT | Gilbert et al., 1980 |
| Fluctuation test | +/- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | NT | NT | Gilbert et al., 1980 |
| Spot test | - | NT | 0 | NT | NT | NT | + | NT | NT | NT | 0 | NT | QR | QR | Seiler, 1973 |
| Plate incorporation | + | 0 | NT | 0 | 0 | 0 | NT | NT | NT | NT | NT | 0 | NT | NT | Geiger and Neal, 1981 |
| Plate incorporation | - | NT | NT | NT | 0 | NT | NT | NT | NT | NT | NT | NT | NT | NT | Geiger and Neal, 1981 |
| Suspension assay | - | NT | 0 | NT | NT | NT | + | NT | NT | NT | NT | NT | NT | NT | Hussain et al., 1972 |
| Suspension assay | +/- | 0 | NT | 0 | 0 | NT | NT | NT | NT | NT | NT | 0 | NT | NT | Zeiger, 1983 |

*The assay was performed under both aerobic and anaerobic conditions.

NT = Not tested; QR = Questionable response

The source of the 2,3,7,8-TCDD sample studied in this paper was the Food and Drug Administration, and its reported purity was 99%. Also, Seiler (1973) observed a positive mutagenic response in a spot test of 2,3,7,8-TCDD performed in the absence of a metabolic activation system. However, the purity of the sample studied was not provided. In tester strains G46 and TA1530, the ratio of revertants/$10^8$ cells in the treated plates divided by spontaneous revertants/$10^8$ cells was <1. In strains TA1531 and TA1534, the ratio was between 1 and 2, which was considered a "doubtful" mutagenic response, while in strain TA1532, the ratio was >10. There was no mention of the 2,3,7,8-TCDD levels tested in this assay. The positive controls, diethylsulfate, 2-aminopurine and 2-aminofluorene, produced ratios of 2-5, <1 and 5-10, respectively, in strain TA1532. In both the study by Hussain et al. (1972) and the study by Seiler (1973), 2,3,7,8-TCDD produced a positive mutagenic response only in the S. typhimurium strain TA1532, which is sensitive to frameshift mutagens.

Hussain et al. (1972) also performed a mutagenicity test of 2,3,7,8-TCDD in two other microbial test systems. A positive response was observed in Escherichia coli Sd-4 as indicated by a reversion to streptomycin independence. In this assay, cells were treated in suspension for 1 hour with 2,3,7,8-TCDD at 0.5-4 μg/mℓ. The greatest mutation frequency (256 mutants x $10^{-8}$, as compared with the control frequency of 2.2 mutants x $10^{-8}$) occurred at a dose level of 2 μg/mℓ. The absolute number of colonies/plate was 7 for the control and 46 for the treated plate. The dose of 2 μg/mℓ caused an 89% decrease in cell survival. In the second test system, the ability of 2,3,7,8-TCDD to increase prophage induction in E. coli K-39 cells was examined. The vehicle control, DMSO, inhibited prophage induction as compared with the untreated controls, while the most effective dose level of 2,3,7,8-TCDD (0.5 μg/mℓ) resulted in an increased prophage induction as compared with the vehicle control, but not as compared with the untreated controls. Hussain et al. (1972) concluded that 2,3,7,8-TCDD was capable of causing increases in the reverse mutation rate in _E_. _coli_ Sd-4 and that 2,3,7,8-TCDD had a weak ability to induce prophage in _E_. _coli_ K-39 cells.

The studies that followed these two early reports of Hussain et al. (1972) and Seiler (1973) failed to detect mutagenic activity of 2,3,7,8-TCDD in _S_. _typhimurium_. Wassom et al. (1978) cited a personal communication from McCann (1978), which reported that 2,3,7,8-TCDD was inactive in both the spot test and plate incorporation assay with _S_. _typhimurium_ strains TA1532, TA1535, TA1537 and TA1538. Doses and other experimental protocols were not mentioned except that the tests were performed both with and without metabolic activation. Gilbert et al. (1980) reported that 2,3,7,8-TCDD gave "substantially negative results" with _S_. _typhimurium_ strains TA98, TA100, TA1530, TA1535, TA1537, TA1538, G46, TA1532, TA1950, TA1975 and TA1978. Both the standard plate incorporation assay and the bacterial fluctuation test were used, and both were performed with and without S-9 prepared from the livers of Aroclor 1254 pretreated rats. In the plate incorporation assay, the test compound was tested at 1-2000 μg/plate under both aerobic and anaerobic conditions. Details were not provided for the fluctuation assay. It is difficult to assess possible reasons for the conflicting results between the earlier studies and these later mutagenicity assays, since information on experimental conditions was limited in the negative studies.

In an attempt to resolve the conflicting results and observe a mutagenic response, Geiger and Neal (1981) tested 2,3,7,8-TCDD in the standard plate incorporation assay using S-9 prepared from different sources. In order to maximize the amount of compound tested, dioxane was used, a better solvent for 2,3,7,8-TCDD than the commonly employed DMSO. Even with the use of dioxane, the limited solubility of 2,3,7,8-TCDD allowed only 20 μg/plate to be tested, a dose that was shown to be non-toxic to the cells. The S-9 used in these assays was prepared from the livers of Aroclor 1254 pretreated male Sprague-Dawley rats and male Golden Syrian hamsters, and from 2,3,7,8-TCDD induced male hamsters. In all assays at 2,3,7,8-TCDD concentrations of 0.2, 2, 5 or 20 μg/plate, and regardless of the source of the S-9, there was no observed mutagenic response. In further attempts to duplicate the previous positive results, Geiger and Neal (1981) tested the same concentrations of 2,3,7,8-TCDD in strain TA1537, a more sensitive direct descendent of strain TA1532, for mutagenic activity in the absence of S-9. Again, no increase in the number of revertants was observed. In assays either with or without S-9, positive controls had predictable increases in the number of revertant colonies. The authors concluded that 2,3,7,8-TCDD was not active under the conditions of this assay; however, testing at higner concentrations may elicit a positive response. It was also noted that many other polychlorinated aromatic compounds are not mutagenic in the Ames test, even though there is positive evidence of carcinogenicity.

Mutagenic effects of 2,3,7,8-TCDD in yeast were observed by Bronzetti et al. (1983). Positive results for reversion and gene conversion were obtained _in vitro_ and in the host-mediated assay. The _in vitro_ experiments yielded small dose-related increases in $trp^+$ convertants and $ilv^+$ revertants. An S10 metabolic activation system was required. Exposure of the yeast to 2,3,7,8-TCDD at the highest level tested (10 μg/mℓ) resulted in 16% survival and yielded 4-fold increases in reversion and gene conversion.

In the host-mediated assay, male mice were exposed to 25 μg of 2,3,7,8-TCDD/kg (Bronzetti et al., 1983). After 5, 10, 20 or 30 days, 0.2 mℓ of a yeast culture ($4x10^{8}$ cells) was instilled retroorbitally. Four hours later, the liver and kidneys were removed and the yeast cells in these organs were assayed for mutagenic responses. Increases (4- to 6-fold) in reversion and gene conversion were observed in yeast cells obtained from the livers and kidneys. The toxic response of the animals to an exposure of 25 μg/kg was not described in this report. The positive results described in this paper may suggest that 2,3,7,8-TCDD is mutagenic in yeast, but more definitive studies are needed before a firm conclusion can be drawn.

Hay (1982) has found that 2,3,7,8-TCDD dissolved in DMSO transformed baby hamster kidney cells, BHK, *in vitro*. The dioxin isomers 2,8-dichloro- and 1,3,7-trichlorodibenzo-p-dioxin also transformed BHK cells, but the response was weak. The unchlorinated dibenzo-p-dioxin and the fully chlorinated octachlorodibenzo-p-dioxin were both negative in the BHK assay (i.e., there was no cell transformation). More recently, Rogers et al. (1982) reported that 2,3,7,8-TCDD induced mutations in the excess thymidine, thioguanine and methotrexate selective systems in L5178Y mouse lymphoma cells in culture.

The National Toxicology Program (Zeiger, 1983) provided data on TCDD from four assay systems: the *S. typhimurium* (strains TA98, TA100, TA1535 and TA1537) histidine reversion assay, the sex-linked recessive lethal test in *Drosophila*, and cytogenetic studies (sister chromatid exchange and chromosome aberrations) in Chinese hamster ovary cells. Negative results were obtained in all of these assays. These studies cannot be evaluated, however, because the procedures used to obtain the data were not described.

The solubility of 2,3,7,8-TCDD in water is only 0.2 μg/ℓ (Crummett and Stehl, 1973). Therefore, negative in vitro results must be viewed with caution unless precise descriptions of the preparation of each test sample are supplied (e.g., were the samples predissolved and, if so, in what solvent).

In vitro reactions of 2,3,7,8-TCDD with bacteriophage QB RNA were evaluated by Kondorosi et al. (1973). Active RNA was purified from QB phage followed by incubation for 1 hour at 37°C with 0.0, 0.2, 2.0 or 4.0 μg/mℓ of 2,3,7,8-TCDD. At all concentrations tested, 2,3,7,8-TCDD had no effect on the transfectivity of QB RNA. Other compounds tested included the alkylating agents methyl, ethyl and isopropyl methanesulfonate, and diethyl pyrocarbonate, all of which inactivated QB RNA under the same experimental conditions. The authors suggested that 2,3,7,8-TCDD inactivity in this assay indicated that 2,3,7,8-TCDD was an intercalating agent, and hence would require double stranded DNA in order to interact. The data presented in this study, however, were insufficient to support this conjecture.

In vivo binding of radiolabeled 2,3,7,8-TCDD to liver macromolecules was studied in Sprague-Dawley rats by Poland and Glover (1979). Both male and female animals were administered [1,6-$^{3}$H] 2,3,7,8-TCDD by intraperitoneal injection at a dose of 7.5 μg/kg. This dose corresponded to a tritium level of 0.87 mCi/kg. The animals were killed 12, 48 and 168 hours after treatment, or 24 hours after treatment when the animals were pretreated with the enzyme inducers phenobarbital or unlabeled 2,3,7,8-TCDD. Following sacrifice, isolation of macromolecules, and removal of free labeled 2,3,7,8-TCDD, the amount of label bound to protein, RNA and DNA was determined. The greatest non-extractable binding of labeled 2,3,7,8-TCDD occurred to protein; however, the amount of label bound was small and only amounted to 0.03-0.1% of the total radioactivity administered. The total amount of label associated with RNA and DNA was, respectively, only 50 and 4 cpm above background. Time after exposure, sex, or prior enzyme induction had no significant effect on 2,3,7,8-TCDD binding. As a result of the extremely low levels of radioactivity associated with RNA and DNA, it is uncertain whether 2,3,7,8-TCDD truly binds covalently to these macromolecules and, if so, whether there is any biological significance to this low level of apparent binding.

The effects of 2,3,7,8-TCDD exposure on the extent of chromosomal aberrations in the bone marrow of male rats were reported in an abstract by Green and Moreland (1975). In the initial experiment, no increase in chromosomal aberration was observed after 5 daily gavage treatments at a 2,3,7,8-TCDD dose of 10 μg/kg. In the second portion of this study, rats were exposed by a single intraperitoneal injection of 2,3,7,8-TCDD at 5, 10 or 15 μg/kg or a single gavage treatment at 20 μg/kg. The animals at the two highest exposure levels were killed 24 hours post-treatment, while the remaining animals were killed 29 days post-treatment. Again, no increase in chromosomal aberrations was observed, except in the positive control group exposed to triethylenemelamine.

In a later report, a small but significant increase in chromosomal aberrations was observed in the bone marrow cells of male and female Osborne-Mendel rats (Green et al., 1977). Bone marrow cells for cytogenetic analysis were obtained from Osborne-Mendel rats used in a range-finding study preliminary to a chronic bioassay (Green et al., 1977). The animals in groups of 8 males and 8 females received twice weekly intubations of 2,3,7,8-TCDD at respective doses of 0.25, 1.0, 2.0 and 4.0, or 0.25, 0.5, 2.0 and 4.0 μg/kg for 13 weeks. Because it was not required for the range-finding study, a control group was not included. Bone marrow cells were analysed for abnormalities and cells in mitosis in the animals that survived to the end of the study (4-8 animals/group). The only significant increases in chromosomal aberrations in comparison with the low dose group were in males at 2 and 4 μg/kg and females at 4 μg/kg. The greatest incidence observed was 4.65% of the cells with chromosomal breaks in the high-dose males, and this was considered only weakly positive. The weak response, as well as the lack of data from control animals and the reported difficulty of obtaining cells from the high-dose animals as a result of 2,3,7,8-TCDD toxicity, makes the conclusion from this study that 2,3,7,8-TCDD produced chromosomal breaks tenuous.

A similar response was observed by Loprieno et al. (1982) in male and female CD-1 mice which received an intraperitoneal injection of 2,3,7,8-TCDD at a dose of 10 μg/kg. At 96 hours post-treatment, there was a significant ($p<0.01$) increase in bone marrow cells with gaps and chromatid aberrations. When chromosomal aberrations were analyzed at 24 hours post-treatment, there was not significant change in the incidence of cells with aberrant chromosomes. The study was continued with a more extensive experiment using CD-COBS female rats. The rats were treated weekly by gavage (vehicle acetone-corn oil 1:6) at doses of 0, 0.01, 0.10 or 1.00 μg/kg for 45 weeks. Analysis of bone marrow cells for chromosomal aberrations 24 hours after the last treatment failed to detect any significant increases.

Czeizel and Kiraly (1976) reported an increased incidence ($p<0.001$) of chromatid-type and unstable chromosome aberrations in the peripheral lymphocytes of workers exposed to the herbicides 2,4,5-trichlorophenoxyethanol (2,4,5-TCPE) and Buminol. The 2,3,7,8-TCDD levels in the final product were <0.1 mg/kg; however, the exposure levels for individual workers were not available.

In a letter, Mulcahy (1980) reported no increased incidences of chromosomal aberrations in the lymphocytes of 15 soldiers exposed to "Agent Orange". The exposure was for 6-15 months and all subjects complained of symptoms, including skin eruptions, which they associated with "Agent Orange". The analyses were performed with lymphocytes obtained ~10 years after the last exposure, and comparisons were made with eight subjects who had no history of exposure to 2,3,7,8-TCDD. Neither sister chromatid exchange nor structural aberrations including both gaps and breaks were increased. The authors note that the long time between exposure and analysis may have accounted for the negative results.

Also, both Reggiani (1980) and Mottura et al. (1981) have studied inhabitants in Seveso, Italy, exposed to 2,3,7,8-TCDD from an accident in a trichlorophenol manufacturing plant. Reggiani (1980) examined 4 adults and 13 children (3-13 years) for chromosomal aberrations within 2 weeks of the accident. These 17 individuals were examined to support claims of, and determine extent of, injury before an inquest judge. Although burn-like skin lesions in these 17 individuals indicated chemical exposure, no increase in chromosomal aberrations was detected. The methods of performing the analyses and the actual number of aberrations detected were not described. Similar negative results were reported in an abstract by Mottura et al. (1981). In this study, subjects were chosen from the area of heavy contamination following the accident (acute high level exposure), from the working population of the plant (chronic low level exposure) and a non-exposed control population. The number of subjects in each group was not provided. The specimens were examined by three independent laboratories and no laboratory reported an increase in chromosomal aberrations, although there was a significant difference in the reported scores between laboratories. There was no information in this abstract on the extent of individual exposure or the length of time that elapsed between the accident and obtaining samples for analyses of chromosomal aberrations.

DiLernia et al. (1982) conducted additional studies on lymphocytes prepared in 1976 and 1979 from eight persons considered acutely exposed to 2,3,7,8-TCDD in the Seveso accident, eight ICMESA factory workers (considered chronically exposed), and 14 control subjects (eight had chromosomes prepared in 1976 and six in 1979). Cells were examined for average number of SAs (evidence for functional ribosomal genes), both on a cell basis and for the large acrocentric chromosomes (D group chromosomes). There was no change in the frequency of SAs on a per cell basis in any of the groups as compared to control values, nor in D group chromosomes from acutely exposed subjects examined immediately after the accident. There was, however, a decrease in the average frequency of SAs in group D chromosomes of acutely exposed subjects examined in 1977 and in ICMESA workers at both the 1976 and 1979 examinations. Although the biologic relevance of these observations has not yet been confirmed, DiLernia et al. (1982) observed a similar decrease in SAs after exposure of lymphocytes to x-irradiation. It was concluded that the decrease in SAs may have resulted from mutagenic damage to functional nucleolar organizing regions.

The potential of exposure to 2,3,7,8-TCDD to result in chromosomal damage has been studied in experimental animals and humans. Most of the studies in experimental animals gave no evidence that 2,3,7,8-TCDD may result in chromosomal aberrations; however, there is a report of a single weak positive response and little detail was provided in the report to assess the quality of the results. In the studies of humans, exposure occurred to chemicals that contain 2,3,7,8-TCDD as a contaminant. In two of these studies involving individuals exposed in the Seveso accident, there was no observed increase in the incidence of chromosomal aberrations. In a third report of individuals exposed at Seveso, changes were observed in lymphocyte chromosomes from exposed workers which were suggested to have risen from mutation in functional nucleolar organizing regions; however, this bioassay has yet to be validated. In the only positive study, workers in a chemical plant were exposed to the herbicides, 2,4,5-TCPE and Buminol, as well as to 2,3,7,8-TCDD. The participation of the herbicides in the resulting increase in the workers of chromosomal aberrations cannot be excluded. At present, the data from experimental animals and humans are too limited to designate 2,3,7,8-TCDD as a clastogenic agent.

In summary, a limited number of initial studies of the mutagenicity of 2,3,7,8-TCDD in bacteria reported positive results in S. typhimurium strain TA1532 in the absence of a mammalian metabolic activation system (Hussain et al., 1972; Seiler, 1973). More recent attempts to repeat these results with strain TA1532 or related strains have failed (Geiger and Neal, 1981; Gilbert et al., 1980; McCann, 1978). These authors have also reported no increase in mutation rate when 2,3,7,8-TCDD was tested in the presence of a mammalian metabolic activation system. In other in vitro assays, 2,3,7,8-TCDD has produced a positive response in reversion to streptomycin independence in E.

*coli* Sd-4 cells and questionable positive response with prophage induction in *E. coli* K-39 cells (Hussain et al., 1972). Also, 2,3,7,8-TCDD has been reported to be mutagenic in the yeast *S. cerevisiae* in both the *in vitro* assay with S-10 and the host-mediated assay (Bronzetti et al., 1983). Rogers et al. (1982) have also reported positive mutagenicity results in the mouse lymphoma assay. In the *E. coli* studies, the poor survival of the cells or the interference of the vehicle solvent, DMSO, with the assay makes the evaluation of the studies difficult. With the data available, it is not possible to resolve the conflicting reports on the mutagenic potential of 2,3,7,8-TCDD.

Overall, the data indicate little potential for the interaction of 2,3,7,8-TCDD with nucleic acids or the ability of 2,3,7,8-TCDD to produce chromosomal aberrations. Kondorosi et al. (1973) demonstrated that 2,3,7,8-TCDD did not react with RNA *in vitro* in the absence of a metabolic activation system. *In vivo* studies using radiolabeled 2,3,7,8-TCDD indicated some association of non-extractable label with RNA and DNA (Poland and Glover, 1979); however, the very low level of bound label observed suggests that the "binding" may have been merely an artifact. Similar marginal data were available on the clastogenic effect of 2,3,7,8-TCDD. Although one *in vivo* study in rats (Green and Moreland, 1975) failed to demonstrate any treatment-related chromosomal aberration, a second study by the same authors (Green et al., 1977) using a longer exposure period reported a small increase in the number of aberrations. In humans exposed to 2,3,7,8-TCDD during the manufacture of 2,4,5-TCPE and Buminol, Czeizel and Kiraly (1976) reported an increase in the number of chromosomal aberrations, while no increase was detected in individuals exposed to 2,3,7,8-TCDD following an industrial accident in Seveso, Italy (Reggiani, 1980; Mottura et al., 1981).

The studies of the clastogenic effect of 2,3,7,8-TCDD were presented with little or no experimental detail to assist in evaluating the merits of the reports. The data available are too limited to indicate whether 2,3,7,8-TCDD can interact with nucleic acids or produce chromosomal aberrations.

The differences among the results described above could be due to several factors, such as treatment protocols, solubility problems, purity of the samples tested and the high toxicity of 2,3,7,8-TCDD. This chemical may be a weak mutagen, but because it is very toxic, the dose range for detecting a positive genetic effect may be very narrow. Therefore, additional experimentation is necessary before any conclusive determination can be made. Suggested further testing includes the ability of 2,3,7,8-TCDD to induce forward mutations in mammalian cells in culture, additional yeast and bacterial studies and the sex-linked recessive lethal test in Drosophila.

Pertinent information regarding the mutagenicity of PeCDDs and HxCDDs were not located in the available literature.

## 5.3. TERATOGENICITY AND OTHER REPRODUCTIVE EFFECTS

**5.3.1. Studies on Human Populations.** A positive association between 2,4,5-T exposures and increases in birth defects or abortions has been reported in human populations in Oregon (U.S. EPA, 1979), New Zealand (Hanify et al., 1981) and Australia (Field and Kerr, 1979). A lack of any such association has been reported in human populations in Arkansas (Nelson et al., 1979), Hungary (Thomas, 1980), New Zealand (Dept. of Health, New Zealand, 1980; McQueen et al., 1977) and Australia (Aldred, 1978). Almost all of the reports are geographic correlation studies, and because of the uncertainties inherent in this type of epidemiologic investigation, as well as the difficulties in distinguishing the effects of 2,4,5-T from those of 2,3,7,8-TCDD contamination, none of the reportedly positive associations unequivocally identify either 2,4,5-T or 2,3,7,8-TCDD as the causative agent. Similarly, the reportedly negative associations do not rule out 2,4,5-T or 2,3,7,8-TCDD as potential teratogens or abortifacients in humans.

Based on a report of a high incidence of abortions in a small group of women living around Alsea, Oregon, who may have been exposed to the herbicide 2,4,5-T from aerial spraying (Smith, 1979), the U.S. EPA (1979) initiated a study, often referred to as the "Alsea II study", to determine if spontaneous abortion rates differed between the exposed and unexposed populations, if spontaneous abortion rates evidenced seasonal variation in these two groups, and if such seasonal variations were associated with 2,4,5-T spray application.

Spontaneous Abortion Rate Index, as defined by the U.S. EPA, is "basically the ratio of the number of hospitalized spontaneous abortions to the number of births corresponding to the spontaneous abortions, based on the residence zip code of the women contributing to each event." Upon completion of the study, the U.S. EPA concluded that: 1) the 1972-1977 Spontaneous Abortion Rate Index for the study area was significantly higher than in the Rural Control Area or the Urban area; 2) there was a statistically significant seasonal cycle in the abortion index in each of the areas with a period of ~4 months, in particular there was an outstanding peak in the study area in June; and 3) there was a statistically significant correlation between the Spontaneous Abortion Rate Index and spray patterns in the study area when a lag-time of 2 or 3 months was included. The U.S. EPA concluded, however, that "This analysis is a correlational analysis, and correlation does not necessarily mean causation."

Milby et al. (1980), citing three critiques of the Alsea II study that were not published in the open literature, state that the statistical method and basic design of the Alsea II study were sufficiently flawed to make this study of no use in human risk assessment. The Alsea II study has also been reviewed by a panel of epidemiologists who, in a published report of their meeting, also concluded that the basic design of the study was inadequate to demonstrate either an effect or absence of an effect of exposure to 2,4,5-T (Coulston and Olajos, 1980). The major inadequacies of the study were that the data collection methods were biased and would likely result in the underestimation of abortions, particularly in the urban area (the incidence of abortions in all three groups was within the expected background rate of 8-15%); only a small part of the area from which the exposed subjects were selected was actually sprayed with 2,4,5-T, and the study was not controlled for other factors such as age, smoking habits and alcohol consumption, which may affect the spontaneous abortion rate. Based on a report by Smith (1979), the U.S. EPA is attempting or has attempted to correlate 2,3,7,8-TCDD levels in the affected areas with the observed rate of abortion. No published reports have been located on the outcome of this effort.

In the only other report encountered on a population in the United States, Nelson et al. (1979) noted a general increase in the reported incidence of facial cleft in both high and low exposure groups in Arkansas from 1948-1974. In this study, exposure estimates were based on average rice production in different areas of Arkansas, and the incidence of cleft palate was determined by screening birth certificates and checking records of the Crippled Children's Services. No consistent exposure/effect correlations were noted, and the general increase with time in the incidence of facial clefts was attributed to better reporting procedures; however, there does not have to be a direct correspondence of malformations in human beings and experimental animals.

Of the four reports available from New Zealand (Dept. of Health, New Zealand, 1980; McQueen et al., 1977; Hanify et al., 1981; Smith et al., 1982a), the report by the Department of Health is essentially anecdotal, involving two women who gave birth to malformed children (one with an atrial septal defect and a malformation of the tricuspid valve of the heart, and the other with biliary atresia). In both cases, exposure to 2,4,5-T could not be ruled out. Based on an analysis of spraying records, the time course of the pregnancies and plant damage near the women's homes, however, the Department of Health, New Zealand (1980) concluded that there was insufficient evidence to implicate 2,4,5-T spraying as a causative factor. Even if the spraying had been implicated, a lack of information on 2,3,7,8-TCDD levels in the spray and the absence of any monitoring data on 2,4,5-T or 2,3,7,8-TCDD would limit the usefulness of this report.

The study by McQueen et al. (1977) is not published in the open literature but is summarized by Milby et al. (1980). According to the summary, McQueen et al. (1977) "...examined the epidemiology of neural-tube defects in three areas in New Zealand and concluded 'there is no evidence to implicate 2,4,5-T as a causal factor in human birth defects.'" No additional details are provided.

Hanify et al. (1981) performed an epidemiologic study in Northland, New Zealand, in areas where spraying of 2,4,5-T was carried out by various companies for a number of years. The rate of birth defects was obtained from an examination of hospital records in seven mutually exclusive areas on a monthly basis over a period extending from 1959-1977. The rate of birth defects from 1959-1965 represented the rate for a non-exposed population since this was prior to the use of 2,4,5-T, while the incidence of birth defects from 1972-1976 represented the rate for the exposed population. During the time of the survey there were 37,751 births, 436 stillbirths, 264 deaths shortly after birth, and 510 congenital anomalies. Three categories of birth defects, heart abnormalities, hypospadias and epispadias, and talipes, had elevated rate ratios of >1 ($p=0.05$) in comparisons between the exposed (1972-1976) and control (1959-1965) populations. Exposure estimates were made for the seven areas and for different years using company records of aerial spraying and a model that factored in assumed fractional removal rates/month (this factor was assumed to be either 1.0 or 0.25). Comparisons of the rate of specific malformations with exposure demonstrated a statistically significant association between the occurrence of talipes and exposure when the fractional removal rate was assumed to be 0.25. There was, however, no statistically significant association where 1.0 was used as the fractional removal rate.

Smith et al. (1982a) investigated the outcome of pregnancy in families of professional 2,4,5-T applicators and agricultural contractors in New Zealand. Agricultural contractors were chosen as the control population since both sprayers and contractors were of the same economic group with similar outdoor occupations. The survey was conducted by mail with 89% of the chemical applicators responding and 83% of the agricultural contractors responding to questions asking whether they used 2,4,5-T and its temporal relationship to reproductive histories regarding birth, miscarriages, stillbirths and congenital defects. The relative risks of congenital defects and miscarriages were 1.19 (0.58-2.45% confidence limits) and 0.89 (0.61-1.30% confidence limits) for the wives of chemical sprayers as compared to the wives of agricultural contractors. These data indicate that exposure of fathers and mothers (e.g., while cleaning clothes) had no effect on the outcome of pregnancy. Biases that may have affected the results, such as the age of the mother at childbirth, smoking habits and birth to Maori parents were investigated and eliminated as possible confounders.

The two reports from Australia (Aldred, 1978; Field and Kerr, 1979) also present apparently conflicting results. The report by Aldred (1978) is not published in the open literature, but the following summary is taken from Milby et al. (1980): "The report concluded that birth defects in a group of babies born in the [Yarram] district in 1974 and 1976 could not be attributed to exposure to 2,4,5-T or 2,4-D." Additional details that might be useful in assessing the rationale for this statement are not provided in the summary. The report by Field and Kerr (1979) plotted the incidence of neural-tube defects (anencephaly and meningomyelocele) in New South Wales, Australia, over the years 1965-1975, and the previous years usage of 2,4,5-T in all of Australia. The authors noted a decrease in the incidence of neural-tube defects expected on the basis of the plotted line in 1975 and 1976, when Australia instituted monitoring of 2,4,5-T to ensure a 2,3,7,8-TCDD level <0.1 ppm. The data were not tested for significance; although Field and Kerr (1979) indicate that they consider the epidemiological data on neural-tube defects to be "relatively complete," they do not comment on the increasing incidence of neural-tube defects during the time period of this study and whether or not an increase in the thoroughness of reporting neural-tube defects could have contributed to the apparent correlation of 2,4,5-T exposure with these defects. A visual replotting of the data suggests that the incidence of cleft palate correlates better with 2,4,5-T usage than with time. Nonetheless, the appropriateness of correlating 2,4,5-T usage in all of Australia with the incidence of defects in one area of Australia is questionable.

Thomas (1980) used an approach similar to that of Field and Kerr (1979) on data from Hungary. One major difference, however, is that Thomas (1980) compared the incidence of all cases of stillbirths, cleft lip, cleft palate, spina bifida, anencephalus and cystic kidney disease in Hungary between 1976 and 1980 with 2,4,5-T use in 1975 in Hungary. Thomas (1980) considered Hungary to be "...probably the best country in which to examine possible health effects of this herbicide" because Hungary requires compulsory notification of malformations diagnosed from birth to age 1 year, a relatively large percentage (55%) of the Hungarian population lives in rural areas where 2,4,5-T exposure may be expected to be greatest, and annual use of 2,4,5-T in Hungary had risen from 46,000 kg in 1969 to 1,2000,000 kg in 1975. In any event, all indices of birth defect rates decreased or remained stable over the period of study.

In addition to contamination of 2,4,5-T being a potential source of 2,3,7,8-TCDD exposure, 2,3,7,8-TCDD is also an inadvertant contaminant of 2,4,5-trichlorophenol (TCP). Chronic exposure to 2,3,7,8-TCDD may occur during the manufacture of TCP and high level acute exposure to 2,3,7,8-TCDD occurred after a July, 1976 accident at the ICMESA TCP chemical factory in Seveso, Italy (Bonaccorsi et al., 1978). In this accident, the reaction used to produce TCP became uncontrolled, producing conditions favorable for 2,3,7,8-TCDD formation prior to venting the contents of the chemical reactor into the atmosphere. The resulting cloud of chemicals settled over a heavily populated area. Although the amount of 2,3,7,8-TCDD released was not known, the reported cases of chloracne, a symptom of acute exposure to

2,3,7,8-TCDD, indicated that exposure had occurred. Some preliminary results are available from epidemiologic studies of reproductive events in the inhabitants of Seveso, and a new study is now available on the reproductive history of men employed in the chemical manufacturing industry with possible chronic exposure to 2,3,7,8-TCDD (Townsend et al., 1982).

Epidemiologic studies to determine the reproductive effects in individuals exposed to 2,3,7,8-TCDD and TCP following the accidental contamination of a populated area around Seveso, Italy, are not completed. The incidence of spontaneous abortions occurring between March, 1976 and January, 1978 have been reported for inhabitants in the area around Seveso by Bonaccorsi et al. (1978), Reggiani (1980) and Bisanti et al. (1980). The spontaneous abortion rate in the contaminated area for the three trimesters following the accident was 13.1, 11.0 and 13.05%, which was similar to the worldwide 15-20% frequency of spontaneous abortion. Subdividing the contaminated area into highly, moderately and least contaminated, and examining the rates for each area individually, also failed to demonstrate any change in the spontaneous abortion rate. The incidence rates of malformations also were examined; however, the numbers were too small for meaningful assessment. There are several reasons why these studies would not indicate that 2,3,7,8-TCDD exposure in this accident was without effect on human reproduction. The authors note that there are many difficulties in interpreting these data. The incidence rates of spontaneous abortions and birth defects were not adequately available for the region prior to the accident as a result of suspected under-reporting. There was inadequate reporting even after the accident due to political turmoil with regard to the management of health services. Also, an unknown number of pregnancies were surgically aborted for fear of 2,3,7,8-TCDD induced birth defects. In a recent review of the progress of epidemiologic investigations of the Seveso accident, Tognoni and Bonaccorsi (1982) indicated that the data on spontaneous abortions and malformation rates still needed verification, and that these data were too preliminary to allow for conclusions.

Townsend et al. (1982) investigated the reproductive history of wives of employees potentially exposed to 2,3,7,8-TCDD during chlorophenol production. A total of 930 potentially exposed males were identified who had worked for ≥1 month between January, 1939 and December, 1975, in a job with potential 2,3,7,8-TCDD exposure. Exposure estimates of low, moderate and high were made by an industrial hygienist primarily from job description and surface contamination data; however, the high potential exposure group was reserved for process workers during 1963-1964 when changes in operations resulted in a number of cases of chloracne. The control population was an equal number of male employees not involved in any process that might cause exposure to 2,3,7,8-TCDD and matched for date of hire. In these groups, 586 wives were identified and 370 agreed to participate as the exposed group, while 345 wives in the control group agreed to participate. After identification of the participants, a personal interview was conducted with the wives to determine pregnancy outcome. Of the 737 total conceptions in the exposed category and 1785 conceptions in the control category (conceptions that occurred in the exposed group prior to work records indicating potential exposure to 2,3,7,8-TCDD were placed in the control group), there was no statistically significant increase in spontaneous abortions, stillbirths, infant deaths or selected congenital malformations. Sample sizes were too small to provide meaningful data if the populations were subdivided by extent of exposure. It was suggested that many confounding factors could account for these negative results, such as the inappropriate selection of the populations, unidentified covariables and insufficient power. However, it was maintained that these results were consistent with animal data, which report that paternal exposure to 2,3,7,8-TCDD does not affect the conceptus.

Poole (1983), in testimony before the House Committee on Science and Technology, described a reanalysis of the primary data used by Townsend et al. (1982). In this reanalysis, the rate of cleft palate and cleft lip were reported to be elevated by 1.9 (90% confidence intervals of 1.0-3.6) in the years 1971-1974 for both the control and exposed groups (the comparison population was not described). At the same House Committee hearing, Houk (1983) presented data from the Birth Defect Monitoring Program of the Center for Disease Control on the yearly rate of cleft palate alone or cleft lip with or without cleft palate for births in Midland County, Michigan during the years 1970-1981. The data indicated an increased rate for these defects of between 50 and 100% in the years 1971-1975, with the rate returning to expected from 1976-1981. The observed increase was only statistically significant if the rates for cleft palate alone and cleft lip with or without cleft palate were combined; however, it was the opinion of Houk (1983) that these defects should not be combined since the causal mechanism may be different. The Michigan Department of Public Health (1983) also reported these results and, in addition, demonstrated that the same results occurred if the comparison was made with other counties in Michigan as well as with the general population of the United States. It was noted in this report that "runs" of increases in oral cleft for successive years have occurred in six other counties with no obvious potential for chemical exposure described. The Michigan Department of Public Health (1983) interpreted the data to indicate that a more detailed case control study was necessary to determine if any common factors may exist, such as exposure to chemicals contaminated with 2,3,7,8-TCDD.

**5.3.2. Studies on Experimental Mammals.** Courtney et al. (1970a,b) were the first to report that 2,4,5-T was capable of causing teratogenic effects in rats and mice. In these studies, rats and two strains of mice were exposed subcutaneously or orally to 2,4,5-T containing 30 ppm 2,3,7,8-TCDD. The mixture was teratogenic and fetotoxic to mice at $\geq$46.4 mg/kg. Rats were more sensitive, exhibiting fetotoxic responses at 10 mg/kg for this 2,4,5-T/2,3,7,8-TCDD mixture. Since this initial report, research has focused on determining the role of 2,3,7,8-TCDD contamination in eliciting the teratogenic response. These studies are summarized in Table 5-22.

Neubert and Dillmann (1972) conducted a detailed study to determine the significance of 2,3,7,8-TCDD contamination. These investigators assayed three 2,4,5-T samples: a highly purified sample containing <0.02 ppm 2,3,7,8-TCDD (referred to as Sample A), a purified sample identical to that used by Roll (1971) that contained 0.05$\pm$0.02 ppm 2,3,7,8-TCDD (Sample B), and a commercial sample containing an undetermined quantity of 2,3,7,8-TCDD (Sample C). All three samples induced cleft palates in NMRI mice at sufficiently high doses. In terms of the number of fetuses with cleft palate/the total number of fetuses, the dose/response pattern observed by Neubert and Dillmann (1972) was similar to that observed by Roll (1971) using a similar grade of 2,4,5-T. In addition to the three 2,4,5-T samples, Neubert and Dillmann (1972) also assayed a sample of 2,3,7,8-TCDD alone and in various combinations with the highly purified sample of 2,4,5-T. This approach allows at least partial quantification of the significance of 2,3,7,8-TCDD contamination in 2,4,5-T-induced cleft palates. When the litter is used as the basic experimental unit, the incidences of cleft palate (number of litters with cleft palate/total numbers of litters) versus the dose can be plotted on log dose/probit response paper, correcting for central response

TABLE 5-22

Studies on the Potential Teratogenic Effects of 2,3,7,8-TCDD-Contaminated 2,4,5-T

| Species/Strain | Vehicle | Form of 2,4,5-T | TCDD Level | Daily Dose | Treatment Days | Observation Day | Maternal Response | Fetal Response | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Mice/NMRI | Rape-seed oil | acid | <0.02 ppm (Sample A) | 8, 15, 30, 45, 60, 90 and 120 mg/kg | 6-15 | 18 | No toxic effects; decreased maternal weight at doses of 90 mg/kg and greater | Significant increases in the incidence of cleft palates at doses above 30 mg/kg (see text for additional details). Significantly decreased ($p<0.005$) fetal weight at all dose levels. | Neubert and Dillmann, 1972 |
| Mice/NMRI | Rape-seed oil | acid | 0.05±0.02 ppm<br>(Sample B) | 30, 60 and 90 mg/kg | 6-15 | 18 | No toxic effects; decreased maternal weight at 90 mg/kg | Increases in the incidence of cleft palate at 60 and 90 mg/kg; significantly decreased ($p<0.005$) fetal weight at all dose levels | Same as above |
| Mice/NMRI | Rape-seed oil | acid | NR (Sample C) | 90 mg/kg | 6-15 | 18 | No toxic effects but decreased maternal weight | Increase in the incidence of cleft palate; significant ($p<0.005$) decrease in fetal weight | Same as above |
| Mice/NMRI | Rape-seed oil | butyl ester | NR | 12 and 17 mg/kg | 6-15 | 18 | No toxic effects | Significant decrease in fetal weight but no effect on mortality; increase in the frequency of cleft palate similar to that seen with acid (see text) | Same as above |
| Mice/NMRI | NR | acid | 0.05±0.02 ppm | 20, 35, 60, 90 and 130 mg/kg | 6-15 | NR | Toxic effects observed at 90 and 130 mg/kg | Increases in the percentage of resorptions and/or dead fetuses at 90 and 130 mg/kg; increases in the incidence of cleft palate and retardation of skeletal development at 35 mg/kg and above | Roll, 1971 |
| Mice/CD-1 | Corn oil:acetone (9:1) | acid | <0.05 ppm | 115 mg/kg | 10-15 | 18 | No significant effect on weight gain or liver-to-body weight ratios | No effect on fetal mortality or fetal weight but an increase in the incidence of cleft palate | Courtney, 1977 |

TABLE 5-22 (cont.)

| Species/Strain | Vehicle | Form of 2,4,5-T | TCDD Level | Daily Dose | Treat-ment Days | Obser-vation Day | Maternal Response | Fetal Response | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Mice/C57BL/6 | Honey:water (1:1) | acid | 30 ppm | 46.4 and 113 mg/kg | 6-14 | 18 | NR | Significant ($p<0.01$) increases in the incidence of cleft palate in the high dose group and cystic kidney in both dose groups; increased fetal mortality also observed in the high dose group | Courtney et al., 1970a,b |
| Mice/AkR | Honey:water (1:1) | acid | 30 ppm | 113 mg/kg | 6-15 | 19 | Increase in liver-to-body weight ratio | Significant ($p<0.05$) increases in the incidence of cleft palate and fetal mortality | Same as above |
| Rat/Sprague-Dawley (groups of 25 rats) | Gavage/hydroxy-propyl-methyl-cellulose | acid | 0.5 ppm | 1, 3, 6, 12 or 24 mg/kg/day | 6-15 | 20 | No effect on body weight and no observable signs of toxicity | A slight but statistically significant ($p<0.05$) decrease in implantations and litter size in lowest dose group only; no frank teratogenic effects based on a detailed examination of the control and 24 mg/kg dose group; the only effect noted was an increase in the incidence of 5th partially ossified sternebrae | Emerson et al., 1970, 1971 |
| Rat/Wistar | Gavage/aqueous gelatin or corn oil | acid | <0.5 mg/kg | 25, 50, 100 or 150 mg/kg/day | 6-15 | 22 | Some maternal mortality and decreased body weight gain at 150 mg/kg; no signs of toxicity at 100 mg/kg or below | At 100 or 150 mg/kg decreased fetal weight, increased fetal mortality and an increase in the incidence of skeletal anomalies; no significant effect at the two lower dose levels | Khera and McKinley, 1972; Khera et al., 1971 |
| Rat/Wistar | Gavage/aqueous gelatin or corn oil | butyl ester | <0.5 mg/kg | 50 or 150 mg/kg/day | 6-15 | 22 | NR | No significant effect on fetal mortality, fetal weight, or the incidence of anomalies | Khera and McKinley, 1971; Khera et al., 1971 |

TABLE 5-22 (cont.)

| Species/Strain | Vehicle | Form of 2,4,5-T | TCDD Level | Daily Dose | Treatment Days | Observation Day | Maternal Response | Fetal Response | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Rat/Holtzman | Gavage/1:1 solution of honey and water | acid | 30 ppm | 4.6, 10.0 and 46.4 mg/kg/day | 10-15 | 20 | NR | Significant ($p<0.01$) increases in fetal mortality at the two higher dose levels; dose related increases in the percent of abnormal fetuses per litter; a high incidence of cystic kidneys in treated groups | Courtney et al., 1970a,b |
| Rat/CD | Gavage/15% sucrose solution | acid | 0.5 ppm | 10.0, 21.5, 46.4 and 80.0 mg/kg/day | 6-15 | 20 | Reduced maternal weight gain at the two higher dose levels ($p<0.05$) and increased liver-to-body weight ratio at the highest dose level ($p<0.05$) | Increase in the incidence of kidney anomalies, but no increase in cleft palate | Courtney and Moore, 1971 |
| Rat/Strain not specified | Gavage/methocel | acid | 0.5 ppm | 50 mg/kg | 6-15 | NS | No effect on mortality or body weight gain | No significant effect on fetal mortality or fetal weight; a significant ($p<0.05$) increase in the incidence of delayed ossification | Sparschu et al., 1971a |
| Rat/Strain not specified | Gavage/methocel | acid | 0.5 ppm | 100 mg/kg | 6-10 | NS | Increased mortality and decreased body weight gain | Increase in the incidence of delayed ossification and poorly ossified or malaligned sternabrae ($p<0.05$) | Same as above |
| Syrian hamster/ Mesocricetus auratus | Gavage/acetone, corn oil and carboxymethyl cellulose in ratio of 1:5.8:10 | acid | <0.1-4.5 ppm | 20, 40, 80 and 100 mg/kg | 6-10 | 14 | NS | Dose-related increases in fetal mortality, gastrointestinal hemorrhages and fetal abnormalities; see text for discussion of effect of TCDD level on development | Collins et al., 1971 |

NS = Not specified; NR = not reported

using Abbott's equation. According to this method, the $ED_{50}$ (by eye-fit) for cleft palate induction are:

2,3,7,8-TCDD: 4.6 μg/kg bw
2,4,5-T (Sample A): 115 mg/kg bw
2,4,5-T (Sample B): 46 mg/kg bw

If the assumption were made that all teratogenic activity in the 2,4,5-T samples were attributable to 2,3,7,8-TCDD contamination, the expected $ED_{50}$ for samples A and B would be 230,000 mg/kg (0.0046 mg/kgx0.02 ppm$^{-1}$) and 92,000 mg/kg (0.0046 mg/kgx0.05 ppm$^{-1}$), respectively. Since the observed $ED_{50}$ was lower by a factor of over 1000, 2,3,7,8-TCDD appears not to be the sole factor in 2,4,5-T-induced cleft palate.

The nature of possible interaction between 2,4,5-T and 2,3,7,8-TCDD is more difficult to define. Based on assays of five mixtures of 2,3,7,8-TCDD and the highly purified 2,4,5-T, Neubert and Dillmann (1972) noted a greater than additive effect on the induction of cleft palates. A similar conclusion can be reached if one assumes that Sample A was a "totally pure" sample of 2,4,5-T. Using the assumptions of simple similar action (Finney, 1971) and treating Sample B as a mixture of 2,3,7,8-TCDD and 2,4,5-T, the expected $ED_{50}$ for Sample B would be 119.8 mg/kg. The observed value of 46 mg/kg again suggests a greater than additive effect. A more detailed statistical analysis of these data, however, would be required to support the assumptions of simple similar action or independent joint action that are implicit in these analyses. Furthermore, the inability to define precisely the levels of 2,3,7,8-TCDD in the 2,4,5-T samples and the possible significance of other contaminants would preclude an unequivocal interpretation of the results of the analysis.

Nevertheless, three of the studies summarized in Table 5-22 (Neubert and Dillmann, 1972; Roll, 1971; Courtney, 1977) have demonstrated the induction of cleft palate in mice by using 2,4,5-T samples containing 2,3,7,8-TCDD levels of 0.05±0.02 ppm or less. Although 2,3,7,8-TCDD contamination is undoubtedly a factor in the teratogenic activity of 2,3,7,8-TCDD contaminated 2,4,5-T, the above analysis suggests that 2,3,7,8-TCDD contamination is not the sole factor, and that some teratogenic activity must be attributed to 2,4,5-T itself or other contaminants in 2,4,5-T.

Effects on reproductive success and fertility have also been studied in four groups of C57BL/6 male mice (25 animals/group) following oral ingestion of mixtures of 2,4-D, 2,4,5-T and 2,3,7,8-TCDD. Daily doses of ~40 mg/kg 2,4-D, 40 mg/kg 2,4,5-T and 2.4 μg/kg 2,3,7,8-TCDD in Group II; 20 mg/kg 2,4-D, 20 mg/kg 2,4,5-T and 1.2 μg/kg 2,3,7,8-TCDD in Group III; and 40 mg/kg 2,4-D, 40 mg/kg 2,4,5-T and 0.16 μg/kg 2,3,7,8-TCDD in Group IV animals were given. Vehicle control animals in Group I had corn oil added to the feed. At the conclusion of an 8-week dosing period, treated animals were mated to untreated virgin females. No significant decrease in fertility, reproduction and germ cell toxicity were noted. Survival of offspring and the development of the newborns apparently were not affected (Lamb et al., 1980).

Courtney and Moore (1971) tested a purified sample of 2,3,7,8-TCDD for teratogenic potential. A summary of this study and others assessing the teratogenic potential of purified 2,3,7,8-TCDD are presented in Table 5-23. CD-1, DBA/2J and C57Bl/6J mice were given subcutaneous injections of 2,3,7,8-TCDD at 1 or 3 μg/kg/day on days 6-15 of gestation in the study by Courtney and Moore (1971). This dose regime did not result in maternal toxicity, although an increase in the maternal liver-to-body weight ratio

TABLE 5-23

Studies on the Potential Teratogenic Effect of 2,3,7,8-TCDD

| Species/Strain | Vehicle | Daily Dose | Treatment Days | Observation Day | Maternal Response | Fetal Response | Reference |
|---|---|---|---|---|---|---|---|
| Mouse/C57BL/6<br>Mouse/AKR | DMSO or honey:water (1:1) | 21.5, 46.4, 113.0 mg/kg | 6-14 or 9-17 | 19[a] | increased liver/body weight ratio | fetocidal, cleft palate, cystic kidney | Courtney et al., 1970b |
| Mouse/CD-1<br>Mouse/DBA/2J<br>Mouse/C57Bl/6J | DMSO | 0.5, 1, 3 µg/kg | 6-15 | 17[a] or 18 | increased liver/body weight ratio | cleft palate, kidney anomalies | Courtney and Moore, 1971 |
| Mouse/C57Bl/6 | Acetone: corn oil (1:9) | 1, 3 µg/kg | 10-13 or 10 | 18[a] | none reported | cleft palate, kidney anomalies | Moore et al., 1973 |
| Mouse/CD-1 | DMSO or corn oil | 25, 50, 100, 200, 400 µg/kg | 7-16 | 18[b] | increased liver/ body weight ratio | cleft palate, hydro-nephrotic kidneys, hydrocephalus, open eyes, edema, petechiae | Courtney, 1976 |
| Mouse/CF-1 | corn oil: acetone (98:2) | 0.001, 0.01, 0.1, 1.0, 3.0 µg/kg | 6-15 | 18[a] | none reported | cleft palate, dilated renal pelvis | Smith et al., 1976 |
| Mouse/NMRI | rape-seed oil | 0.3, 3.0, 4.5, 9.0 µg/kg | 6-15 | 18 | no effect observed | fetocidal at the high dose, cleft palate at doses at or above 5 µg/kg | Neubert and Dillmann, 1972 |
| Rat/CD | DMSO | 0, 0.5, 2.0 µg/kg | 6-15, 9 and 10, or 13 and 14 | 20[a] | none reported | kidney malformations at both dose levels | Courtney and Moore, 1971 |
| Rat/Sprague-Dawley | corn oil/ acetone | 0, 0.03, 0.125, 0.5, 2.0 and 8.0 µg/kg | 6-15 | 20[a] | vaginal hemorrhage at 2.0 and 8.0 µg/kg | intestinal hemorrhage at 0.125 and 0.5 µg/kg, fetal death at higher doses, subcutaneous edema | Sparschu et al., 1971b |
| Rat/Wistar | corn oil/ anisole | 0.0, 0.125, 0.25, 1, 2, 4, 8, 16 µg/kg | 6-15 | 22 | maternal toxicity observed at or above 1 µg/kg | increased fetal death observed at or above 1 µg/kg, subcutaneous edema and hemorrhages in the 0.25-2 µg/kg groups | Khera and Ruddick, 1973 |

TABLE 5-23 (cont.)

| Species/Strain | Vehicle | Daily Dose | Treatment Days | Observation Day | Maternal Response | Fetal Response | Reference |
|---|---|---|---|---|---|---|---|
| Rat/Sprague-Dawley | corn oil/ acetone (9:1) | 0.1, 0.5, 2.0 μg/kg | 1-3 | 21 | decrease in body weight gain in the high dose group | decreased fetal weight in the 0.5 and 2 μg/kg group | Giavini et al., 1982a |
| Rat/Sprague-Dawley | diet | 0.001, 0.01 and 0.1 μg/kg[c] | throughout gestation | post-parturition | low fertility at 0.01 and 0.1 μg/kg decreased body weight at 0.01 and 0.1 μg/kg dilated renal pelvis | low survival at 0.01 and 0.1 μg/kg, decreased body weight at 0.01, slight dilated renal pelvis at 0.001 μg/kg in the $F_1$ but not succeeding generations | Murray et al., 1979 |
| Rabbit/ New Zealand | corn oil/ acetone (9:1) | 0.0, 0.1, 0.25, 0.5 and 1 μg/kg | 6-15 | 28 | maternal toxicity at doses of 0.25 μg/kg and above | increases in extra ribs and total soft tissue anomalies | Giavini et al., 1982b |

[a] First day of gestation designated day zero

[b] First day of gestation designated day one

[c] The high dose level (0.1 μg/kg/day) was discontinued due to very low fertility in adults

was observed in DBA/2J and C57Bl/6J mice. 2,3,7,8-TCDD had no measurable effect on fetal mortality; however, anatomical abnormalities were observed in all strains and at all dose levels, with C57Bl/6J being the most sensitive strain. The abnormalities observed were cleft palate and unspecified kidney anomalies.

Moore et al. (1973) treated pregnant C57Bl/6 mice with an oral dose of 2,3,7,8-TCDD at 1 or 3 μg/kg/day on days 10-13 of gestation, or 1 μg/kg on day 10 of gestation. At the high dose level, the average incidence of cleft palate was 55.4%. Kidney anomalies (hydronephrosis) were observed on an average of 95.1% of the fetuses/litter, with 83.1% having bilateral kidney anomalies. When the dose was decreased to 1 μg/kg/day, the average incidence of cleft palate dropped to 1.9%; however, the incidence of kidney anomalies remained relatively high, with an average incidence of 58.9%. On the average, bilateral kidney anomalies occurred in 36.3% of the fetuses/litter. A single dose of 1 μg/kg on day 10 of gestation produced kidney anomalies in 34.3% of the fetuses; however, no cleft palates were observed. When C57Bl/6 mice were treated with 1 μg/kg on day 10 of gestation and were then allowed to litter, the detection of kidney lesions on postnatal day 14 was found to depend largely on whether the pups nursed on a 2,3,7,8-TCDD-treated mother. When pups from a 2,3,7,8-TCDD-treated mother nursed on control mice, kidney anomalies were found in only 1/14 litters. In contrast, when pups from control mothers nursed on 2,3,7,8-TCDD-treated mice, kidney anomalies were observed in 4/14 litters. In the pups exposed to 2,3,7,8-TCDD both _in utero_ and during the postnatal period, kidney anomalies were observed in 5/7 litters. Kidney anomalies observed following _in utero_ exposure or exposure through the milk were similar, and these kidney anomalies may not be considered a purely teratogenic response.

Neubert et al. (1973) reviewed what was known of the embryotoxic effects of 2,3,7,8-TCDD in mammalian species. Also reported were their own studies and previous work (Neubert and Dillmann, 1972) using NMRI mice, in which cleft palate was observed to be a common abnormality; however, no kidney anomalies were reported. Neubert and Dillmann (1972) administered 2,3,7,8-TCDD by gavage to 20 female mice on days 6 through 15 of gestation at doses of 0.3, 3.0, 4.5 and 9.0 μg/kg. At day 18 of gestation, extensive reabsorption was observed in the high dose group with 6/9 litters totally resorbed. In the few surviving fetuses, there was an 81% incidence of cleft palate. At lower doses, there were 9 and 3% incidences at doses of 4.5 and 3.0 μg/kg, respectively, and no cleft palates were observed in 138 fetuses examined in the 0.3 μg/kg group. Fetal mortality was increased at the 9.0 μg/kg dose if animals were treated only on days 9 through 13; however, the incidence of cleft palate remained high at a frequency of 60%. In a series of experiments to determine the time of gestation at which 2,3,7,8-TCDD was effective in inducing cleft palate, mice were treated for a single day between days 7 and 13 of gestation with 2,3,7,8-TCDD at a dose of 45 μg/kg. A maximum number of inducted cleft palates occurred when animals were treated on either day 8 or 11 of gestation, while exposure to 2,3,7,8-TCDD after day 13 of gestation produced no cleft palates in the fetuses.

Courtney (1976) compared the teratogenic potential of 2,3,7,8-TCDD administered orally with 2,3,7,8-TCDD administered subcutaneously. CD-1 mice were dosed with 2,3,7,8-TCDD on days 7 through 16 of gestation at levels of 25, 50, 100, 200 or 400 μg/kg/day; the 400 μg/kg dose was not used in animals treated by subcutaneous injection. Doses of 200 or 400 μg/kg/day produced vaginal bleeding and high rates of abortion. A dose of 100 μg/kg/day was fetotoxic, resulting in decreased fetal weight and survival. Anatomic abnormalities were observed at all dose levels, with cleft palate and hydronephrotic kidneys being most common. Other abnormalities observed included hydrocephalus, open eye, edema and petechiae. Subcutaneous administration of 2,3,7,8-TCDD produced a greater teratogenic response at a lower dose than oral administration, with abnormalities observed in 87% of the fetuses following subcutaneous administration and 42% after oral administration of a dose of 25 μg/kg/day.

The effects of 2,3,7,8-TCDD on the incidence of fetal anomalies were also studied by Smith et al. (1976) in CF-1 mice. The mice were given 0.001-3.0 μg 2,3,7,8-TCDD/kg/day by gavage from day 6 through 15 of gestation. The incidence of cleft palate was found to be significantly increased in 1.0 and 3.0 μg/kg/day dose groups, and the incidence of kidney anomalies was significantly increased at 3.0 μg/kg/day. There were no observable teratogenic effects in the study at 0.1 μg/kg/day; however, some were noted at lower dose levels, although not statistically significantly elevated.

Poland and Glover (1980) compared cleft palate formation by 2,3,7,8-TCDD in the responsive C57BL/6J, the non-responsive DBA/2J and the hybrid B6D2F1/J strains of mice. Female mice were mated with male mice of the same genetic strain, and on day 10 of pregnancy the pregnant mice were given a single subcutaneous dose of 3.0, 10.0 or 30.0 μg/kg of 2,3,7,8-TCDD dissolved in p-dioxane or the solvent (control) alone (0.4 mℓ/kg). On day 18, the animals were killed and the number of cleft palates and resorbed fetuses was determined. At doses of 3.0 and 10.0 μg/kg of 2,3,7,8-TCDD, cleft palates (3% incidence among live fetuses) were observed only in the C57BL/6J mice at the higher dose level. At a dose of 30 μg/kg, the incidence of cleft palates among live fetuses for the C57BL/6J, B6D2F1/J and

DBA/2J mice was 54, 13 and 2%, respectively. This study also reported that cleft palate formation was significantly higher in several other responsive mouse strains compared with non-responsive mice. At a dose level of 30 µg/kg of 2,3,7,8-TCDD, the incidence of cleft palates among live fetuses for the responsive C57BL/6J, A/J, BALB/cByJ and SEC/1REJ mice was 54, 73, 65 and 95%, respectively. The only responsive mouse (CBA/J) strain that was resistant to 2,3,7,8-TCDD-mediated cleft palate was also resistant to the teratogenic effects of cortisone. In contrast, the incidence of cleft palates in the non-responsive DBA/2J, RF/J, AKR/J, SWR/J and 129/J mice was between 0-3% at the 30 µg/kg dose level. Thus the responsive mice, containing high levels of the Ah receptor, are highly susceptible to the effects of 2,3,7,8-TCDD in producing cleft palate, whereas the non-responsive mice, which contain low (or 0) levels of the Ah receptor protein, are resistant to this teratogenic effect of 2,3,7,8-TCDD. These data and other results (Hassoun and Dencker, 1982) suggest that cleft palate formation elicited by 2,3,7,8-TCDD segregates with the Ah locus.

In an early study, Courtney and Moore (1971) tested the teratogenic potential of 2,3,7,8-TCDD in pregnant rats (CD) injected subcutaneously on a daily basis with 2,3,7,8-TCDD (0.5 or 2 µg/kg) in DMSO on days 6 through 15, days 9 and 10, or days 13 and 14 of gestation. The only remarkable anomaly was kidney malformations in fetuses exposed to 2,3,7,8-TCDD. In the group exposed transplacentally at a dose of 0.5 µg/kg, 4/6 litters had fetuses with kidney malformations (average number of kidney defects/litter was 1.8). An 11 and 34% incidence of kidney anomalies occurred in groups exposed to 2,3,7,8-TCDD on days 9 and 10, and 13 and 14, respectively. In addition, six hemorrhagic gastrointestinal tracts were observed in the treated group (these data were not enumerated with respect to dose); however, this was considered a primary fetotoxic effect of 2,3,7,8-TCDD and not a malformation.

2,3,7,8-TCDD was administered by gavage to groups (10-14 animals/group) of pregnant Sprague-Dawley rats at dose levels of 0, 0.03, 0.125, 0.5, 2.0 and 8.0 μg/kg/day on days 6 through 15 of gestation (Sparschu et al., 1971b). No adverse teratogenic effects were reported in fetuses exposed transplacentally at the 0.03 μg/kg level. At the 0.125 μg/kg level, three dead fetuses were reported, fetal weights were slightly depressed, and intestinal hemorrhage was noted in 18 of 127 examined fetuses. In the group given doses of 0.5 μg/kg, the number of viable fetuses was reduced, resorptions were increased, 6 dead fetuses were reported, and 36 of 99 fetuses suffered an intestinal hemorrhage. In the 2.0 μg/kg group, only 7 live fetuses were reported (occurring in only 4/11 litters), 4 having intestinal hemorrhage. Early and late resorptions were prevalent. No live fetuses, but many early resorptions, were reported in the group exposed to 8.0 μg 2,3,7,8-TCDD/kg/day. Subcutaneous edema appeared dose-related, occurring in a considerable number of fetuses from the higher dose groups. Male fetuses appeared to be more susceptible to 2,3,7,8-TCDD exposure; however, there was no significant difference in the sex ratio of live fetuses.

Khera and Ruddick (1973) tested a wide range of doses of 2,3,7,8-TCDD for teratogenic and fetotoxic potential. Groups of 7-15 Wistar rats were intubated with 2,3,7,8-TCDD at doses of 0.125, 0.25, 1, 2, 4, 8 and 16 μg/kg on days 6 through 15 of gestation. At day 22 of gestation, there were no live fetuses in groups exposed to ≥4 μg/kg, and reduced litter size was observed in the 1 and 2 μg/kg group. Unspecified maternal toxicity was reported in all groups where there was fetal mortality. In groups exposed to 0.25-2 μg/kg, there were fetal anomalies observed as either gross or microscopic lesions consisting of subcutaneous edema of the head and neck, and hemorrhages in the intestine, brain and subcutaneous tissue. The incidences of grossly observed lesions were 0/18, 2/11, 7/12 and 11/14 in the control, 1, 1 and 2 μg/kg dose groups, respectively (the study was conducted in two parts, and the 1 μg/kg dose was repeated). With regard to the other dose levels tested, the table enumerating the results had an entry of "not done". The incidence of microscopically observed lesions for the control, 0.25, 0.5, 1, 1 and 2 μg/kg groups was 0/10, 1/33, 3/31, 3/10, 3/6 and 3/7, respectively. There were no effects of treatment observed in the 0.125 μg/kg group.

Khera and Ruddick (1973) also exposed dams to 2,3,7,8-TCDD at doses of 0.125, 0.25, 0.5 and 1 μg/kg on days 6 through 15 of gestation and allowed the dams to litter and wean the pups. In this experiment, maternal toxicity was reported in the 0.5 and 1 μg/kg group. At birth, there were fewer viable pups, and the pups had lower body weight in all but the 0.125 μg/kg group. At weaning on day 21 after birth, there were no surviving pups in the 1 μg/kg group, and 40% of the pups in the 0.5 μg/kg group did not survive. Fostering pups from dams exposed to 2,3,7,8-TCDD at 1 μg/kg onto control dams did not appreciably increase survival, while fostering control pups onto dams exposed to 2,3,7,8-TCDD did not increase pup mortality. These data suggest that poor pup survival was a result of delayed toxicity from in utero exposure to 2,3,7,8-TCDD.

Giavini et al. (1982a) assessed the effect of small doses of 2,3,7,8-TCDD administered during the preimplantation period in Sprague-Dawley rats. The animals, in groups of 20, were treated by gavage with 2,3,7,8-TCDD at doses of 0.0, 0.1, 0.5 and 2 μg/kg on days 1-3 of gestation. (The legends to the tables in this paper indicated that the low dose was 0.125 μg/kg.)

At day 21 of gestation, no toxic effects were observed in the dams except for a decrease from 19.3-12.9 g in average maternal weight gain in the high dose animals as compared with controls. In the fetuses, weight was significantly reduced ($p<0.05$) in the 0.5 and 2 μg/kg groups. Malformed litters and malformation/fetuses examined were 2, 5, 5 and 6, and 2/270, 8/260, 5/255 and 8/253, respectively, in the control 0, 0.1, 0.5 and 2 μg/kg groups; however, these increases in the treated animals were not statistically significant. The anomalies observed were restricted to cystic kidney. This exposure to 2,3,7,8-TCDD early in pregnancy did not affect implantation frequency, and the decrease in fetal weight was considered a result of 2,3,7,8-TCDD delayed implantation.

In a second study, Giavini et al. (1983) administered the same doses of 2,3,7,8-TCDD (0.0, 0.125, 0.5 and 2 μg/kg) daily to 15 female CRCD rats per group by gavage in corn oil:acetone (9:1) for 2 consecutive weeks prior to mating. Females that did not become pregnant during three estrous cycles were necropsied to determine signs of toxicity, while pregnant animals were allowed to proceed to day 21 of gestation, at which time necropsies were performed with particular emphasis on reproductive organs and reproductive success. At the lowest dose tested (0.125 μg/kg), there were no overt clinical signs of toxicity in the dams or adverse effects in any of the fetal parameters examined. At the 0.5 and 2 μg/kg levels, average maternal weight was decreased. Also, one animal in each of these groups did not become pregnant, although necropsy did not reveal any obvious dysfunctions. The only other overt sign of toxicity was listlessness during the treatment period in the animals of the high-dose group. The only significant ($p<0.01$) fetal effect observed in the 0.5 μg/kg group was an increase in post-implantation losses from 2.9% in the control group to 10.2%. In the high-dose group, there were decreases in corpora lutea and implantations (averages of 17.6% in control and 14.9% in treated animals, and 15.5% in control and 12.0% in treated animals, respectively), and increases in both pre- and post-implantation losses of 11.7% for controls and 19.5% ($p<0.05$) in treated animals, and 2.9% in control and 30.3% ($p<0.001$) in treated animals, respectively. In addition to these signs of fetal toxicity, 9 of 10 litters in the high-dose group contained at least one malformed fetus as compared with 1/13, 2/13 and 2/13 in the control, 0.125 and 0.5 µg/kg groups. The predominant fetal malformations were cystic kidney and dilated renal pelvis, which have been observed in other studies in which 2,3,7,8-TCDD was administered during gestation.

The reproductive effects of 2,3,7,8-TCDD were also examined in a 3-generation study using Sprague-Dawley rats (Murray et al., 1979). Throughout the study, animals were continuously maintained on diets providing doses of 0, 0.001, 0.01 or 0.1 µg 2,3,7,8-TCDD/kg/day. The parental group ($f_0$) was maintained for 90 days on the test diets prior to mating. The $f_0$ rats were mated twice, producing the filial generations ($f_{1A}$ and $f_{1B}$). Selected $f_{1B}$ and $f_2$ rats were mated at ~130 days of age to produce the $f_2$ and $f_3$ litters, respectively. In later generations, the high dose group (0.1 µg 2,3,7,8-TCDD/kg/day) was discontinued because few offspring were produced in this group. At the intermediate dose (0.01 µg/kg/day), 2,3,7,8-TCDD caused lower body weight in exposed rats of both sexes ($f_1$ and $f_2$). At the low dose, no toxic effects were discerned.

Fertility was greatly reduced in the $f_0$ generation exposed to 0.1 µg 2,3,7,8-TCDD/kg/day. At 0.01 µg 2,3,7,8-TCDD/kg/day, fertility was significantly ($p<0.05$) reduced in the $f_1$ and $f_2$ rats. Fertility in rats (of any generation) exposed to 0.001 µg 2,3,7,8-TCDD/kg/day was not different from that of control rats. Decreases in litter size were noted in the $f_{1A}$ group exposed to 0.1 μg/kg/day and the $f_2$ and $f_3$ litters exposed at 0.01 μg/kg/day. Statistically significant decreases in fetal survival throughout gestation were noted in $f_2$ and $f_3$ litters of the 0.01 μg 2,3,7,8-TCDD/kg/day exposed dams. At 0.001 μg 2,3,7,8-TCDD/kg/day, a decreased gestational survival was reported for the $f_2$ litters, but not for other generations. Decreased neonatal survival was noted among $f_{1A}$ and $f_2$ pups exposed to 0.01 μg 2,3,7,8-TCDD/kg/day, but not among $f_{1B}$ or $f_3$ pups. Postnatal bw of the $f_2$ and $f_3$ litters at 0.01 μg 2,3,7,8-TCDD/kg/day were significantly depressed. At the low dose (0.001 μg 2,3,7,8-TCDD/kg/day), necropsy of 21-day-old pups revealed a statistically significant ($p<0.05$) increase in dilated renal pelvis in the $f_1$ generation. Subsequent generations at this dose level or any at the intermediate dose (0.01 μg 2,3,7,8-TCDD/kg/day) did not have a significant increase in this abnormality. Significantly decreased thymus weight and increased liver weight were reported in the $f_3$ generation, but not in the $f_1$ generation ($f_2$ generation data not obtained) of the intermediate dose group. Murray et al. (1979) concluded that 2,3,7,8-TCDD ingested at 0.01 or 0.1 μg/kg/day impaired reproduction among rats, and NOAELs were associated with 0.001 μg 2,3,7,8-TCDD/kg/day. Nisbet and Paxton (1982) reevaluated the primary data of Murray et al. (1979) using different statistical methods. From this reevaluation it was concluded that 2,3,7,8-TCDD significantly reduced the gestational index, decreased fetal weight, and increased liver-to-body weight ratios and the incidence of dilated renal pelvis in both lower dose groups. Nisbet and Paxton (1982) concluded that the dose of 0.001 μg/kg/day was not a NOAEL in this study.

A single report by Giavini et al. (1982b) describes the effects of exposure to 2,3,7,8-TCDD on fetal development in rabbits. Groups of 10-15 New Zealand rabbits were administered 2,3,7,8-TCDD by gavage at doses of 0.0, 0.1, 0.25, 0.5 and 1 μg/kg on days 6 through 15 of gestation. The dams were examined for implantation sites, resorptions and live fetuses, and the fetuses were examined for malformations on day 28 of gestation. Decreased maternal weight gain and unspecified signs of maternal toxicity occurred in dams exposed to 2,3,7,8-TCDD at doses of $\geq$0.25 μg/kg. At doses of 0.5 and 1 μg/kg, there were 2 and 4 deaths, respectively, among the dams. There were increases in abortions and resorptions at a dose of $\geq$0.25 μg/kg, with no live fetuses detected in the high dose group. In the fetuses, the most common observation was a significant increase in extra ribs from 33.3% in the controls to 82, 66.6 and 82% in the 0.1, 0.25 and 0.5 μg/kg dose groups. Although there was no significant increase in specific soft tissue anomalies, there was an increase from 0/87-3/78, 2/33 ($p<0.05$) and 2/28 ($p<0.05$) in total soft tissue anomalies in the control, 0.1, 0.25 and 0.5 μg/kg groups. The most prevalent soft tissue anomaly was hydronephrosis, which the authors point out was a common finding in rat fetuses exposed to 2,3,7,8-TCDD *in utero*. These effects were considered to be signs of embryotoxicity rather than a teratogenic effect.

In addition to the fetotoxic effects of prenatal exposure to 2,3,7,8-TCDD, Norman et al. (1978) demonstrated that 2,3,7,8-TCDD could induce liver microsomal enzymes following *in utero* exposure. Pregnant New Zealand rabbits were given subcutaneous injections of 2,3,7,8-TCDD at a dose of 30 nmol/kg (9.6 μg/kg) on day 24 of gestation, and the livers of newborns were examined for enzyme activity within 12 hours after birth. While this treatment increased the liver cytochrome P-450 levels in the adults ~2-fold, from 1.8-3.7 nmol/mg protein, the increase in the newborns was ~5-fold, from 0.3-1.6 nmol/mg protein. SDS-polyacrylamide gel electrophoresis revealed that 2,3,7,8-TCDD induced a single form (form 6) of cytochrome P-450, and that this form was one of the two that were also induced by 2,3,7,8-TCDD in the adult liver. The identity of form 6 was confirmed by immunologic reaction and its peptide fingerprint. It was shown that induction of cytochrome P-450 in newborns resulted in levels of benzo(a)pyrene hydroxylase and 7-ethoxy-resorufin-O-deethylase activity similar to adult levels. The consequence to the newborn of these changes in the development of liver microsomal enzymes has not been established.

Dougherty et al. (1975) failed to find evidence of teratogenicity or embryotoxicity in rhesus monkeys that were given on days 22-38 of gestation daily oral doses (in gelatin capsules) of up to 10 mg/kg/day of 2,4,5-T containing 0.05 ppm 2,3,7,8-TCDD. The 2,3,7,8-TCDD dose at the highest dose level of 2,4,5-T administered (10 mg/kg/day) would correspond to 0.5 μg 2,3,7,8-TCDD/kg/day. However, it should be noted that palate closure in the monkey occurs on gestational days 42-44 and the kidney is also a late developing organ.

Adverse effects of exposure to 2,3,7,8-TCDD on reproductive success in monkeys have also been described. Schantz et al. (1979) fed a diet containing 50 ppt 2,3,7,8-TCDD to rhesus monkeys for 20 months. Seven months into the study the female monkeys were bred to control males. There were four abortions and one stillbirth, two monkeys did not conceive even though they were mated repeatedly, and two monkeys carried their young to term. The total 2,3,7,8-TCDD intake over the 7 months was estimated by the authors to be 0.35 μg/kg, corresponding to a calculated daily dose of 0.0015 μg 2,3,7,8-TCDD/kg/day.

Allen et al. (1979) fed adult female rhesus monkeys on diets containing 50 or 500 ppt of 2,3,7,8-TCDD for 7 months. These exposure levels correspond to total doses per animal at the end of 7 months of 1.8 and 11.7 μg 2,3,7,8-TCDD. Although menstrual cycles were not affected in either treatment group, 5/8 animals in the high dose group had decreased serum estradiol and progesterone levels. Hormone levels were normal in the low dose animals. At 7 months, the females were bred with non-exposed males, and 6/8 and 3/8 females in the low and high dose groups, respectively, were impregnated. Of the impregnated animals, 4/6 and 2/3 had spontaneous abortions, while the remaining impregnated animals had normal births.

McNulty (U.S. EPA, 1980c) treated pregnant rhesus monkeys by gastric gavage to 2,3,7,8-TCDD in a vehicle of corn oil:acetone solution. Group I animals were administered a total dosage of 5 μg/kg bw (two animals), 1 μg/kg bw (four animals) and 0.2 μg/kg bw (four animals) in nine divided doses, 3 times/week during weeks 4, 5 and 6 (days 20 through 40) after conception. Group II, consisting of 12 animals, received single doses of 1 μg/kg bw of 2,3,7,8-TCDD on days 25, 39, 35 and 40 after conception. Three animals were exposed in each of these 4 days. The vehicle control group, consisting of 11 animals, was treated with corn oil:acetone only, on the same schedule as Group I animals. Both of the females that received the highest dose (5 μg/kg) had fetal losses. In the next lower-dosed animals (1 μg/kg in both groups), 12 of 16 females had fetal losses; and in the lowest-dosed animals (0.2 μg/kg in Group I), one abortion occurred in four pregnancies. Maternal toxicity was observed in many of these treated females. The difference in frequency of fetal loss between all pregnant animals given 1 μg/kg and the rate of historical abortion in the author's breeding colony was found to be significant. The author concluded that short exposure to 1 μg/kg bw of 2,3,7,8-TCDD during early pregnancy results in fetal loss in rhesus monkeys and the results appear to be related to the adverse effects of 2,3,7,8-TCDD on the fetus (U.S. EPA, 1980c).

In addition to 2,3,7,8-TCDD, the teratogenic potential of a related chlorinated dibenzo-p-dioxin compound, hexahclorodibenzo-p-dioxin (HxCDD), has been investigated in rats. Pregnant Sprague-Dawley rats were treated by gavage with 0.1, 1.0, 10 or 100 μg HxCDD/kg/day on days 6-15 of gestation (Schwetz et al., 1973). Treatment with high levels of HxCDD (10 and 100 μg/kg) was highly lethal to fetuses during late gestation. There was a significant dose-related increase in late resorptions from 0% (at 0.1 μg/kg/day) to 79% (at 100 μg/kg/day). Weight and length of surviving fetuses were decreased due to HxCDD. The incidences of cleft palate, subcutaneous edema, malformed vertebrae and split sternebrae were significantly increased in fetuses of rats treated with 100 μg HxCDD/kg/day. No increase in fetal anomalies was noted in fetuses exposed to 0.1 μg HxCDD/kg, and only subcutaneous edema was more prevalent in groups exposed at 1 or 10 μg HxCDD/kg/day when compared with controls.

Pertinent information regarding the teratogenicity or reproductive effects of PeCDDs was not located in the available literature.

## 5.4. CHRONIC TOXICITY

Data regarding the toxicity of 2,3,7,8-TCDD have been summarized recently by Kociba and Schwetz (1982) and by Poland and Knutson (1982). The symptoms produced in humans from chronic exposure to 2,3,7,8-TCDD are known from occupational exposure and from accidents that exposed segments of the general population to 2,3,7,8-TCDD contaminated chemicals. These symptoms include weakness and pain with nerve conduction abnormalities, emotional disorders, neuropsychiatric syndromes, eye irritation, hyperlipidaemia, cutaneous hyperpigmentation and chloracne (IARC, 1978). Chloracne was the most commonly observed effect associated with 2,3,7,8-TCDD exposure. Although these symptoms of 2,3,7,8-TCDD exposure have been determined from a number of epidemiology studies, the exposure always occurred in conjuction with other chemicals, as a result of 2,3,7,8-TCDD being a contaminant. Also, quantitative exposure data were not available from these studies, and as a result, the epidemiology studies cannot be used for quantitative risk assessment.

Subchronic and chronic animal studies to assess the carcinogenicity of 2,3,7,8-TCDD have provided limited information on the non-cancer systemic toxicity of this compound (Table 5-24). In an early study, Van Miller et al. (1977a) defined the dietary level of 2,3,7,8-TCDD that adversely affected the longevity of rats following chronic exposure. Groups of 10 male Sprague-Dawley rats were maintained for 78 weeks on diets containing 1, 5, 50, 500, 1000, 5000, 50,000, 500,000 or 1,000,000 ppt of 2,3,7,8-TCDD. Survival was monitored during the study or at termination 95 weeks after initiation of treatment. No animals survived until the end of the study at the five highest exposure levels. The respective week after the start of treatment in which the first death occurred in these high dose groups was 31, 31, 3, 2 and 2 weeks, with all animals in groups >50 ppb dead by week 4. The mortality rate in the 0.0, 1, 5, 50 and 500 ppt groups at 95 weeks was 60, 20, 40, 40 and 50%. Although the small number of animals in each group makes it impossible to precisely define a dose-response relationship, it was apparent that exposure >1 ppb curtailed survival.

TABLE 5-24

Effects of Chronic Exposure to 2,3,7,8-TCDD in Laboratory Rodents

| Species/ Strain | Sex/No. | Dose | Treatment Schedule | Duration of Study | Parameters Monitored | Effects of Treatment | Reference |
|---|---|---|---|---|---|---|---|
| Rat/Sprague-Dawley | M/10 | 0.0 ppt | NA | 95 weeks | survival | 40% survived until 95 weeks, the first death occurred at week 68 | Van Miller et al., 1977a |
| | M/10 | 1 ppt | continuous in diet for 78 weeks | 95 weeks | survival | 80% survived until 95 weeks, the first death occurred at week 86 | Same as above |
| | M/10 | 5 ppt | continuous in diet for 78 weeks | 95 weeks | survival | 60% survived until 95 weeks, the first death occurred at week 33 | Same as above |
| | M/10 | 50 ppt | continuous in diet for 78 weeks | 95 weeks | survival | 60% survived until 95 weeks, the first death occurred at week 69 | Same as above |
| | M/10 | 500 ppt | continuous in diet for 78 weeks | 95 weeks | survival | 50% survived until 95 weeks, the first death occurred at week 17 | Same as above |
| | M/10 | 1000 and 5000 ppt | continuous in diet for 78 weeks | 95 weeks | survival | No animals survived until 95 weeks, the first death occurred at week 31 | Same as above |
| | M/10 | 50,000, 500,000 and 1,000,000 ppt | continuous in diet for 78 weeks | 95 weeks | survival | No animals survived until 95 weeks, the first deaths occurred at weeks 2 and 3 | Same as above |
| Rat/Sprague-Dawley | M&F/50&50 | ~2193 ppt (0.1 µg/kg/day) | continuous in diet for 2 years | 2 years | extensive histopathology, hematology, urine analyses, and clinical chemistry | Cumulative mortality increased (F); Body weight gain decreased (M,F); Red blood cell count decreased (M,F); Packed cell volume decreased (M,F); Hemoglobin decreased (M,F); Reticulocytes increased (M,F); White blood cell count decreased (F); SGPT increased (F); G-Glutamyl transferase increased (F); Alkaline phosphatase increased (F); Urinary coproporphyrin increased (F); Urinary uroporphyrin increased (F); Urinary 4-aminolevulinic acid increased hepatic degeneration increased (M,F) | Kociba et al., 1978, 1979 |

TABLE 5-24 (cont.)

| Species/ Strain | Sex/No. | Dose | Treatment Schedule | Duration of Study | Parameters Monitored | Effects of Treatment | Reference |
|---|---|---|---|---|---|---|---|
| Rat/Sprague-Dawley | M&F/50&50 | ~208 ppt (0.01 μg/kg/day) | continuous in diet for 2 years | 2 years | extensive histopathology, hematology, urine analyses and clinical chemistry | Urinary coproporphyrin increased (F);<br>Urinary uroprophyrin increased (F);<br>Hepatic degeneration increased (M,F) | Kociba et al., 1978, 1979 |
| Rat/Sprague-Dawley | M&F/50&50 | ~22 ppt (0.001 μg/kg/day) | continuous in diet for 2 years | 2 years | extensive histopathology, urine analyses and clinical chemistry | No differences from values obtained from control animals | Same as above |
| Rat/Osborne-Mendel | M&F/75&75 | 0.0 μg/kg/week | NA | 106 weeks | extensive histopathology | Toxic hepatitis; 0/74 (M), 0/75 (F) | U.S. DHHS, 1980b |
| Rat/Osborne-Mendel | M&F/50&50 | 0.5 μg/kg/week | administered by gavage biweekly for 104 weeks | 107 weeks | extensive histopathology | Toxic hepatitis; 14/50 (M), 32/50 (F) | Same as above |
| Rat/Osborne-Mendel | M&F/50&50 | 0.05 μg/kg/week | administered by gavage biweekly for 104 weeks | 107 weeks | extensive histopathology | The incidence of toxic hepatitis was not elevated 0/50 (M), 1/50 (F) | Same as above |
| Rat/Osborne-Mendel | M&F/50&50 | 0.01 μg/kg/week | administered by gavage biweekly for 104 weeks | 107 weeks | extensive histopathology | The incidence of toxic hepatitis was not elevated 1/50 (M), 0/50 (F) | Same as above |
| Mice/B6C3F1 | M&F/75&75 | 0.0 μg/kg/week | NA | 105-106 weeks | extensive histopathology | Toxic hepatitis; 1/73 (M), 0/73 (F) | Same as above |
| Mice/B6C3F1 | M&F/50&50 | 0.5 μg/kg/week (M)<br>2.0 μg/kg/week (F) | administered by gavage biweekly for 104 weeks | 107 weeks | extensive histopathology | Toxic hepatitis; 44/50 (M), 34/47 (F) | Same as above |
| Mice/B6C3F1 | M&F/50&50 | 0.05 μg/kg/week (M)<br>0.2 μg/kg/week (F) | administered by gavage biweekly for 104 weeks | 107 weeks | extensive histopathology | The incidence of toxic hepatitis was not elevated 3/49 (M), 2/48 (F) | Same as above |
| Mice/B6C3F1 | M&F/50&50 | 0.01 μg/kg/week (M)<br>0.04 μg/kg/week (F) | administered by gavage biweekly for 104 weeks | 107 weeks | extensive histopathology | The incidence of toxic hepatitis was not elevated 5/44 (M), 1/50 (F) | Same as above |

TABLE 5-24 (cont.)

| Species/ Strain | Sex/No. | Dose | Treatment Schedule | Duration of Study | Parameters Monitored | Effects of Treatment | Reference |
|---|---|---|---|---|---|---|---|
| Mice/Swiss | M/38 | 0.0 μg/kg/week | NA | 588 days | histology on all organs | Dermatitis and amyloidosis; 0/38 | Toth et al., 1978, 1979 |
| Mice/Swiss | M/44 | 0.007 μg/kg/week | administered by gavage weekly for 1 year | 649 days | histology on all organs | Dermatitis and amyloidosis; 5/44 | Same as above |
| Mice/Swiss | M/44 | 0.7 μg/kg/week | administered by gavage weekly for 1 year | 633 days | histology on all organs | Dermatitis and amyloidosis; 10/44 | Same as above |
| Mice/Swiss | M/43 | 7.0 μg/kg/week | administered by gavage weekly for 1 year | 424 days | histology on all organs | Early mortality, dermatitis and amyloidosis; 17/43 | Same as above |

NA = Not applicable

In the more extensive studies of Kociba et al. (1976, 1978) in rats, U.S. DHHS (1980b) in rats and mice, and Toth et al. (1978, 1979) in mice, extensive histologic examinations were performed. In all of these studies, the predominant histopathology reported was adverse changes in the liver. These changes consisted of fatty degeneration and were observed at doses as low as 0.01-0.07 μg/kg/week in rats (Kociba et al., 1976, 1978; U.S. DHHS, 1980b) and mice (U.S. DHHS, 1980b). In addition, Kociba et al. (1976, 1978) observed increases in urinary prophyrins at a dose of 0.01 μg/kg/week and changes in hematologic parameters at 0.1 μg/kg/week. The only other effects reported after chronic oral exposure of rodents to 2,3,7,8-TCDD were dermatitis and amyloidosis in the kidneys, liver and spleen of Swiss mice (Toth et al., 1978, 1979). The incidence of these lesions were dose-related with incidences of 0/38, 5/44, 10/44 and 17/43 in groups administered 0, 0.007, 0.7 and 7.0 μg/kg/week. Only in the low dose group (0.007 μg/kg/week) in the study by Kociba et al. (1976, 1978) were there no observed effects of treatment.

Allen et al. (1977) maintained eight female rhesus monkeys for 9 months on diets containing 500 ppt 2,3,7,8-TCDD. Deaths occurred in five animals between 7 and 12 months. Other signs of toxicity included hair loss, swollen eyelids and facial edema, while hematologic examination revealed anemia (occurring by 6 months) and pancytopenia (occurring by 9 months). In addition, the autopsies revealed extensive hemorrhage of internal organs, particularly the stomach, hypocellularity of the bone marrow and lymph nodes, and hyperplasia and metaplasia of the epithelium in a number of organs.

There are limited data on the subchronic and chronic toxicity of HxCDD available from a U.S. DHHS (1980c) bioassay assessing the carcinogenicity of a 1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD. In the subchronic study, groups of 10 male and 10 female Osborne-Mendel rats and $B6C3F_1$ mice were used. The rats received doses of 0, 2.5, 5, 10, 50 and 100 μg/kg/week, while the mice received 1.25, 2.5, 5, 10 and 50 μg/kg/week of HxCDD in corn oil:acetone weekly by gavage for 13 weeks. The rats had a dose-related decrease in weight gain, with males appearing to be slightly more sensitive than the females. All groups of mice had poorer weight gain than the control groups, although there was no apparent relationship to dose. Histologic examinations were performed at the termination of the study on 9 male and 10 female rats, respectively, in the 5 and 10 μg/kg/week groups and in 10 male and 9 female mice in the 10 μg/kg/week group, with only selected animals in the remaining groups examined. In rats, ~50% of the males and females in the 5 and 10 μg/kg/week groups, respectively, had signs of threshold to moderate hepatotoxicity, while splenic hyperplasia was observed in the 50 and 100 μg/kg/week groups (~50% of the animals examined) and atrophy of the thymus was detected in 50% of the males and females examined in the 50 μg/kg/week group. In mice, ~50% of the animals had threshold to moderate hepatotoxicity in the 10 μg/kg/week group, and at the 50 μg/kg/week dose the effects were described as "significant histopathologic changes in the liver". No effects were observed in male mice administered 1.25 μg/kg/week or in female mice administered 1.25 or 2.5 μg/kg/week.

In the chronic portion of the U.S. DHHS (1980c) study, male and female Osborne-Mendel rats and $B6C3F_1$ mice were administered by gavage in corn oil:acetone a 1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD for 104 weeks and were followed for an additional 3-4 weeks post-exposure. The groups consisted of 75 males and 75 females for the vehicle controls and 50 males and 50 females in the treatment group. Both male and female rats and male mice received doses of 0.0, 1.25, 2.5 and 5.0 μg/kg/week, while female mice received doses of 2.5, 5.0 and 10 μg/kg/week. The treated male and female rats had a dose-related decrease in body weight gain during the latter portion of the study, and the high dose females had reduced survival. No gross signs of toxicity were observed in mice of either sex. Although extensive histologic examinations were performed, the only treatment-related effect was "toxic hepatitis", which was defined as "degenerative hepatocytic changes and/or necrosis associated with mild fibrosis and infiltration". The incidence of this lesion in control, low, medium and high dose groups, respectively, was, in male rats: 0/75, 28/48, 35/50 and 34/48; in female rats: 0/73, 33/50, 37/50 and 44/50; in male mice: 0/75, 28/50, 35/50 and 34/49; and in female mice: 0/75, 33/50, 37/50 and 44/50. The severity of the "toxic hepatitis" was dose-related; however, it is unclear how severely the liver was damaged at any of the doses. In rats and mice, all doses of this mixture of 1,2,3,6,7,8-and 1,2,3,7,8,9-HCDD represented FELs for liver toxicity.

Pertinent data regarding the chronic toxicity of PeCDD were not located in the available literature.

## 5.5. OTHER RELEVANT INFORMATION

2,3,7,8-TCDD strongly affects microsomal drug metabolizing enzymes in both the liver and kidneys (IARC, 1978). The enzymes induced can both increase and suppress the metabolism of many other foreign compounds (Hook et al., 1975a). Berry et al. (1977) observed increases in the enzyme AHH in both hepatic and extra-hepatic tissues of female rats and fetuses following an intraperitoneal injection of 0.2-5 µg/kg of 2,3,7,8-TCDD on day 17 of gestation. The enhancement of AHH activity in fetal tissue was associated with an increased capacity to metabolize benzo(a)pyrene, PB, with the result that greater DNA binding of BP occurred after the *in utero* exposure to 2,3,7,8-TCDD. Similar results were observed by Lucier et al. (1975b) after administration of 3 µg/kg of 2,3,7,8-TCDD to pregnant rats on days 5, 10 or 16 of gestation. Enzyme induction occurred during late gestation and continued until after birth. It was demonstrated that postnatal exposure of the pups continued to occur after birth through the ingestion of the maternal milk.

Poland et al. (1979) employed the effective dose for the induction of AHH activity in chicken embryo livers in an effort to determine the structure activity relationship among 13 positional isomers of congeners of the PCDD. Four isomers, 2,3,7,8-tetra-, 1,2,3,4,7-penta-, 1,2,3,4,7,8-hexa- and 2,3,7,8-TCDD were active, while the 1,3,6,8- and 1,2,3,4-TCDD along with the mono-, di- and octa-chlorodibenzo-*p*-dioxins were inactive. The authors suggested that biologic activity was imparted to those isomers which have at least three of the lateral ring positions (i.e., those at 2, 3, 7 or 8) chlorinated and had one vicinal ring position free of halogen. Only two dose levels, 470 and $47 \times 10^{-11}$ mol/egg, were used in this study, and it is not clear whether the isomers reported as inactive would have induced AHH activity at higher dosages (a slight increase in activity was observed with 1,2,4,6,7,9-HxCDD at $470 \times 10^{-11}$ mol/egg). Similar structure activity relations were observed by Bradlaw and Casterline (1979) and Bradlaw et al. (1980) in an *in vitro* mammalian cell culture system using rat hepatoma cells. The $ED_{50}$ for induction of AHH activity occurred at doses between 1.5 and >500,000 pmol/plate, with the decreasing order of biologic activity being 2,3,7,8-$Cl_4$, 1,2,3,7,8-$Cl_5$, 1,2,3,4,7,8-$Cl_6$, 1,2,3,6,7,8-$Cl_6$, 1,2,3,7,8,9-$Cl_6$, 1,3,7,8-$Cl_4$, 1,2,3,8-$Cl_4$, 1,2,3,6,7,9-$Cl_6$ and 1,2,4,7,8-$Cl_5$. It is apparent from these data that the structure/activity pattern is analogous to that proposed by Poland et al. (1979) and that the position of the chlorine on the rings was more critical for eliciting biologic activity than was the number of chlorines present. Similar relationships were observed in the ability of congeners of PCDDs to produce keratinization *in vitro* in a mouse teratema cell line (Knutson and Poland, 1980).

Carlstedt-Duke (1979) isolated a protein receptor for 2,3,7,8-TCDD from the cytosol of rats, and Poland and Glover (1979) demonstrated the specific binding of 2,3,7,8-TCDD to the cytosol fraction of mice that were sensitive to 2,3,7,8-TCDD induction of AHH activity. Binding to the cytosol fraction did not occur in strains of mice insensitive to the induction of AHH activity by 2,3,7,8-TCDD. Poland et al. (1979) compared the ability of different congeners of PCDDs to bind specifically to mouse liver cytosol fractions and induce AHH activity in the chicken embryo liver. A strong correlation between these two assays of biologic activity was observed, and it was suggested that the induction of AHH activity (and toxicity) was mediated through this specific binding protein. The "dioxin receptor" was present in many organs in varying concentrations (Carlstedt-Duke, 1979; Okey et al., 1980). The level of the receptor was highest in the thymus, and this may be one factor accounting for the fact that the lymphatic system and the thymus are target organs in the toxicity of 2,3,7,8-TCDD, at least in the guinea pig (McConnell et al., 1978).

The structure-activity relationships among the PCDDs for binding to the cytosol receptor and the induction of AHH (as well as other enzyme) activity corresponds to the structure-activity relationships noted for toxicity. This suggests that the unmetabolized PCDDs are responsible for toxicity, and that the toxic effects are mediated by binding to the cytosol receptor (Poland and Knutson, 1982). There is evidence that these structure-activity relationships are operating through genetic components of susceptibility.

# 6. AQUATIC TOXICITY

## 6.1. ACUTE

The effects of acute exposure to 2,3,7,8-TCDD have been reported for several species of freshwater fish. Juvenile coho salmon, Oncorhynchus kisutch, had an $LC_{50}$ of 0.0056 μg/ℓ (5.6 ppt) following a 96-hour exposure, although manifestation of this mortality required 60 days (Miller et al., 1979). Exposure for 96 hours to 0.0011 μg/ℓ (1.1 ppt) had no effect over a period of 114 days after the beginning of the exposure. A similar exposure of northern pike, Esox lucius, eggs yielded a delayed $LC_{50}$ of 0.001 μg/ℓ following a 96-hour exposure (Helder, 1980); in this instance the mortality was observed by 23 days from the beginning of the exposure. In a subsequent study, Helder (1981) reported that 96 hours of exposure of rainbow trout, Salmo gairdneri, eggs to 0.0001 μg/ℓ (0.1 ppt) resulted in significant growth retardation for 72 days. Norris and Miller (1974) reported that exposure of guppies, Poecilia reticulata, for 120 hours to 0.10 μg/ℓ resulted in 8% mortality during the exposure period and 100% delayed mortality by 37 days after the beginning of the exposure. These studies indicate that acute exposure to as low as 0.0001 μg/ℓ (0.1 ppt) causes delayed toxic effects in fish.

Pertinent data regarding the effect on any other aquatic species of acute exposure to PCDDs were not located in the available literature.

## 6.2. CHRONIC

The effects of chronic or subchronic exposure to 2,3,7,8-TCDD have been reported for three species of freshwater invertebrates and three species of freshwater fish. Miller et al. (1973) exposed snails, Physa sp., worms, Paranais sp. and mosquito larvae, Aedes aegypti, to 0.20 μg/ℓ (200 ppt) for 36, 55 and 17 days, respectively. Reproduction by snails and worms was inhibited; however, there was no effect on mosquitoes by 30 days after beginning the exposure.

Subchronic exposure caused 100% mortality to channel catfish, Ictalurus punctatus, after 20 days at 0.0042 μg/ℓ (4.2 ppt) and 100% mortality to mosquitofish, Gambusia affinis, after 15 days at 0.0028 μg/ℓ (Yockim et al., 1978). The only other information concerning chronic exposure to 2,3,7,8-TCDD in fish was provided in a feeding study with rainbow trout by Hawkes and Norris (1977), who reported that 2300 μg/kg in feed was lethal after 70 days of feeding, but no effect was seen over a 105-day period of diets containing 2.3 μg/kg.

Pertinent data regarding the effects on any other aquatic species of chronic exposure to PCDDs were not located in the available literature.

## 6.3. PLANT EFFECTS

Pertinent data regarding the toxicity of PCDDs to aquatic plants were not located in the available literature.

## 6.4. RESIDUES

2,3,7,8-TCDD has been reported in the edible portions of channel catfish, carp, yellow perch, small mouth bass, suckers and lake trout from the Tittabawassee, Grand and Saginaw Rivers, Lake Michigan and the Saginaw Bay in Michigan at concentrations ranging from <4-695 ppt. The highest concentrations were detected in bass, perch and suckers (Harless and Lewis, 1982). 2,3,7,8-TCDD was found also at concentrations ranging from 15 to >10,000 ppt in turtle eggs and liver, edible fish flesh, snake liver and muscle, catfish, frog liver and muscle, predatory fish and bottom feeder fish (Mitchum et al., 1980). The maximum concentration was reported to be present in frog liver and muscle.

One series of microcosm experiments (Isensee and Jones, 1975; Yockim et al., 1978; Isensee, 1978) provided bioconcentration data for 2,3,7,8-TCDD with algae, *Oedogonium cardiacum*, snails, *Physa* sp., *Helisoma* sp. and cladocerans, *Daphnia magna*. The data for each species were obtained over a range of exposure concentrations from 1.33-0.00005 μg/ℓ (1330-0.05 ppt). BCFs tended to be higher in the midrange concentrations, declining at either extreme, but with few exceptions the bioconcentration estimates were within an order of magnitude and averaged ~4400 after the 30- to 33-day exposure period.

The U.S. EPA (1984) estimated the weighted average BCF for 2,3,7,8-TCDD to be 5000. The derivation of this estimate was presented in Section 2.4. of this report.

# 7. EXISTING GUIDELINES AND STANDARDS

## 7.1. HUMAN

Early guidelines set forth by the National Academy of Sciences Committee on Drinking Water and Health (NAS, 1977) established an ADI for 2,3,7,8-TCDD of $10^{-4}$ µg/kg/day. This ADI was based on a subchronic NOAEL for toxicity in the rat of 0.01 µg/kg/day (Kociba et al., 1976) and was established prior to information on the carcinogenicity of 2,3,7,8-TCDD. Hexachlorophene with no more than 0.1 ppm 2,3,7,8-TCDD is limited to 0.05 ppm in or on cottonseeds used for livestock feed (40 CFR 180.302). For 2,3,7,8-TCDD the U.S. EPA's Carcinogen Assessment Group (CAG) has estimated 95% upper-limit potency to be $1.56\times10^{5}$ $(mg/kg/day)^{-1}$ and the ambient water quality criterion has been recommended to be $1.3\times10^{-7}$ µg/ℓ at an assumed incremental cancer risk level of $10^{-5}$ (U.S. EPA, 1984b). CAG has recommended 11 $(\mu g/kg/day)^{-1}$ as the upper-limit potency estimate, derived from hepatocellular carcinoma and adenoma data in male mice, for a mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin although estimates from other studies range from 11 $(\mu g/kg/day)^{-1}$ to 0.59 $(\mu g/kg/day)^{-1}$ (U.S. EPA, 1984a). The National Research Council of Canada (NRCC, 1981a) has estimated a level of 2,3,7,8-TCDD between 2.1 and 6.3 pg/day for an individual at a lifetime incremental cancer risk level of $10^{-6}$. Canadian regulatory officials concluded that the consumption of one meal of fish containing 20 ppt 2,3,7,8-TCDD should not result in any sufficient health effects (NRCC, 1981a).

The Food and Drug Administration (U.S. DHHS, 1983) advises that fish containing >50 ppt should not be consumed and those containing >25 ppt, but <50 ppt, should not be consumed more than twice a month. The Centers for Disease Control (U.S. DHHS, 1984) has chosen 1 ppb of 2,3,7,8-TCDD in residential soil as a level of concern.

## 7.2. AQUATIC

The ambient water quality criteria document for 2,3,7,8-TCDD (U.S. EPA, 1984b) states:

> Not enough data are available concerning the effects of 2,3,7,8-TCDD on aquatic life and its uses to allow derivation of national criteria. The available information indicates that acute values for some freshwater animal species are >1.0 μg/ℓ; some chronic values are <0.01 μg/ℓ, and the chronic value for rainbow trout is <0.001 μg/ℓ. Because exposures of some species of fishes to 0.01 μg/ℓ for <6 days resulted in substantial mortality several weeks later, derivation of aquatic life criteria for 2,3,7,8-TCDD may require special consideration. Predicted BCFs for 2,3,7,8-TCDD range from 3000-900,000, but the available measured BCFs range from 390-13,000. If the BCF is 5000, concentrations >0.00001 μg/ℓ should result in concentrations in edible freshwater and saltwater fish and shellfish that exceed levels identified in a U.S. FDA health advisory. If the BCF is >5000 or if uptake in a field situation is greater than that in laboratory tests, the value of 0.00001 μg/ℓ will be too high.

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